UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA DE LISBOA

INTERVENTIONS AND OUTCOMES IN BRONCHIOLITIS CLINICAL TRIALS

RICARDO MIGUEL RIBEIRO MARQUES CUNHA FERNANDES

Orientadores:

Prof. Doutor Martin Offringa

Prof. Doutor José Costa Trindade

Prof. Doutora Cristina Sampaio

Tese especialmente elaborada para obtenção do grau de

Doutor em Medicina, especialidade de Pediatria

LISBOA | 2015

UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA DE LISBOA

INTERVENTIONS AND OUTCOMES IN BRONCHIOLITIS CLINICAL TRIALS

RICARDO MIGUEL RIBEIRO MARQUES CUNHA FERNANDES

Orientadores:

Prof. Doutor Martin Offringa

Prof. Doutor José Costa Trindade

Prof. Doutora Cristina Sampaio

Tese especialmente elaborada para obtenção do grau de Doutor em Medicina, especialidade de Pediatria

Júri

Presidente: Prof. Doutor Rui Victorino

Vogais:

Prof. Doutora Johanna Hanneke van der Lee

Prof. Doutora Maria Inês de Azevedo

Prof. Doutor Martin Offringa

Prof. Doutor José Costa Trindade

Prof. Doutora Maria do Céu Machado

Prof. Doutora Teresa Bandeira

Prof. Doutor António Vaz Carneiro

LISBOA | 2015

A impressão desta tese foi aprovada pelo Conselho Científico da Faculdade de Medicina de Lisboa em reunião de 28 de Abril de 2015.

Printing of this thesis was approved by the Scientific Council of Faculdade de Medicina de Lisboa on April 28 2015.

INTERVENTIONS AND OUTCOMES IN BRONCHIOLITIS CLINICAL TRIALS

Ricardo M R M C Fernandes

INTERVENTIONS AND OUTCOMES IN BRONCHIOLITIS CLINICAL TRIALS

PhD thesis, Faculdade de Medicina, Universidade de Lisboa

Ricardo Miguel Ribeiro Marques Cunha Fernandes
rmfernandes@campus.ul.pt

The research described in this thesis was supported by: a PhD research grant from the Gulbenkian Program for Advanced Medical Education (1st edition, 2008); the Cochrane Incentives Funding Scheme (UK Cochrane Centre); a grant from Sociedade Portuguesa de Pediatria. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Cover: BellySketcher by Inês Pargana (http://bellysketcher.com/)

Colhe
todo o oiro do dia
na haste mais alta
da melancolia.
Eugénio de Andrade

Raquel, Henrique: to you, with you, for us.

TABLE OF CONTENTS

PREFACE

This thesis compiles a series of individual manuscripts and related analyses which encompass the topics of definition of acute bronchiolitis, evidence on the safety and efficacy of common interventions, and selection of outcomes and measurement instruments. It is preceded by a general overview on the nature and impact of this common condition in infants. At the time of writing (March 2015) two of the four individual manuscripts (presented in Chapter 2) have been published in peer reviewed journals, while the remaining two (Chapters 3 and 4) have been accepted for revision. My contribution to all projects that led to these manuscripts included developing the research ideas and research questions, designing the studies, writing the protocol and analysis plan, performing the data extraction, conducting all statistical analyses, writing and submitting all manuscripts; and responding to reviewers' comments. The work in this thesis was conducted between 2009 and 2014, under the supervision of Professors Martin Offringa, Cristina Sampaio, and José Costa Trindade.

As I reflect on this journey, I can't help to think that there is a gap between what these pages hold, and the twists and turns of the path that led me here. The thorough and comprehensive standardized language of reporting scientific manuscripts which I absolutely support as an integral part of rigorous and responsible research conduct, can ironically feel dry and meagre to express the many uncertainties and hurdles that went together with each project. Little will be stated here of the immense privilege but also tense challenge of working collaboratively, of the countless days spent dwelling in labyrinthine datasets, and of the almost acrobat-like need to balance science, clinics and personal life. Even if it was described, it would likely sound excessive in face of what is, pragmatically, only a small and temporary increment in broader scientific knowledge in this field. Ultimately, however, as Georges Perec alluded to in a dramatically different context, you would likely learn more of what was experienced from the blank spaces between the lines than in the words written here.

My starting point was determined by previous experiences and circumstance, even as decisive moments along the journey looked strikingly stochastic. Throughout the medical course I was given the opportunity to taste the flavors of basic research in

such diverse fields as biochemistry, embryology and, later, cognitive neuroscience. From Professors Domingos Henrique, Leonor Parreira and Alexandre Castro-Caldas, with whom I had the privilege to work, I learned the need to balance creativeness and rigor when turning ideas into testable scientific hypotheses, the meticulousness and patience of experimental work, and most importantly, the drive to never stop questioning what seems well established. These lessons seemed to fade as I grew more interested in clinical science, and only late did I realize how much common ground there was between them, and how invaluable they were as I moved from bench to bedside, from the molecule to the individual and the population.

The genuine pleasure of practicing pediatrics was soon to come, as was a growing interest in how scientific evidence was produced and appraised. Rather than the often clichéd discussion on the virtues and sins of the so-called "evidence-based medicine", the focus was on the foundations, limits and applicability of evidence and quantitative medicine, and how it intertwined with experience and the patient perspective. An exploratory project on the placebo effect in childhood migraine with Professors Cristina Sampaio and Joaquim Ferreira introduced me to the principles of clinical pharmacology. Training at the Erasmus Program in Rotterdam provided a background on population epidemiology and the fascinating world of causal inference. The spark of methodology finally led me to a fellowship with Professors Martin Offringa and Hanneke van der Lee in Amsterdam, which was pivotal in opening doors to the realm of clinical epidemiology. The gaps in child health evidence were taking centre stage in this field, and initiatives were being established to improve the design, conduct and reporting of clinical trial research in children. As I embarked on this moving train, small yet decisive actions were being taken in Portugal to provide clinical research opportunities for residents, and a doctoral thesis became a natural next step.

From inception, I gravitated towards the topic of wheezing disorders, particularly acute bronchiolitis, with the crucial mentorship from Professors Teresa Bandeira and José Costa Trindade. In hindsight, this choice wasn't just driven by clinical interest in respiratory disease. There is more than meets the eye to the apparently monotonous seasonal bronchiolitis, as this condition reflects some of the challenges of practicing and researching in child health. First, it is a paradigm of an acute pediatric condition that is usually benign but encompasses a wide range of

severities and leads to uncertainties about long-term prognosis in the young developing child. Second, it evokes the contrast between the art of clinical assessment by experienced clinicians, and the quantification of measurable parameters such as physiological variables and clinical scales. Third, while known to every child health practitioner, acute bronchiolitis stands at a crossroads of different subspecialties and settings. Both inevitably entail different perspectives on its definition, on which interventions are useful, and on which outcomes are clinically relevant. Finally, it is also a showcase for the collision between strong opinion-based practice and limited high-quality evidence, with scarce innovation despite its impact for families and health care systems.

A defining moment in the journey to this doctoral thesis was to come in mid-2008, as I joined the first edition of the Gulbenkian Program for Advanced Medical Education, directed by Professor Leonor Parreira and later Professors António Coutinho and Jorge Soares. This pioneering initiative in the portuguese panorama of medical research included a 6-month full time curriculum of intensive education in a vast array of cutting-edge fields of biomedicine, with a highly qualified international faculty. At times both informative and provocative, the program was invaluable in how it incited students to think outside the box of everyday clinical reasoning, to raise their standards of research to a higher level, and to expand horizons in an era of interdisciplinary -omics and systems biology. Faced with the promises and caveats of personalized medicine, it became apparent to me how important it is to have a dialogue between the mechanistic approaches of innovative translational science and the methodological rigor of clinical epidemiology, in order to ensure clinical relevance and minimize waste in clinical research endeavors.

As I resumed my clinical training while pursuing the thesis projects, I faced the challenges of maintaining course and motivation despite the many obstacles of this part-time schedule. I was privileged to have full support from my doctoral program, from the Directors of the Department of Pediatrics at Lisbon's Academic Medical Centre (Professors João Gomes-Pedro, Paulo Ramalho and Maria do Céu Machado), from the Laboratory of Clinical Pharmacology and Therapeutics at the University of Lisbon and Instituto de Medicina Molecular (Professors Cristina Sampaio and Joaquim Ferreira), from all my clinical colleagues and obviously from all three supervisors. Further, a strong ongoing collaboration was established with Professors

Lisa Hartling, Terry Klassen and Amy Plint in Canada, which was paramount to all thesis projects. Opportunities to participate in various research initiatives soon appeared, from the Standards for Research in Child Health (StaR Child Health) to the Cochrane Collaboration Child Health Field, and later also the Core Outcome Measures in Effectiveness Trials (COMET). These activities were time-intensive but rarely distracting, and contributed decisively to the methodological approaches of this thesis' projects. Further, it has been invaluable to participate in the design, conduct and reporting of high-level international collaborative research projects, and, most importantly, these experiences provide perspectives for future postdoc activities.

As discussed throughout this thesis, there are few certainties when predicting the developmental trajectories of wheezy children and infants after acute bronchiolitis. While tempting, a one-size-fits-all-approach to manage these children has repeatedly failed, and some have called it one of the last true art forms in medicine. It is hard to avoid the analogy with the paths of clinicians from medical doctoral programs such as the ones I participated in. The outcome of our own trajectories are likely determined by our background, the nature of our projects, our local settings and ultimately, our drive and personality. The part-time model has enormous challenges in the absence of a research-friendly environment and structure, one that is both fair and rigorous in how it values research work and output as well as clinical skills and productivity, be it during residency or after obtaining certification. The risks of failing at either clinics or research, or worst, to come short at both, cannot be ignored. Protracted projects are also another consequence, one that I endured as this thesis seemed at times to drag as "obras de Santa Engrácia", endlessly at works. But lessons learned from what I have experienced from successful approaches in Canada, Netherlands or the United Kingdom, highlight how this effort of placing research as a core integrated value of the health care system is paramount. As I finish writing this thesis, the decisive circumstances which Portugal is currently facing, call for determination and vision in defining a bearing for clinical research. New opportunities are presenting to clinician researchers, but there is a long road ahead to leverage our assets and produce creative, competitive and meaningful patient-centered research, based on research infrastructures that are fit for purpose. The experience gained during this journey will hopefully contribute to this effort.

This thesis was written as an intersection between clinics and methodology, as well as pediatric practice and science. It reflects the guidance and work of a number of mentors and collaborators to whom I thank in the acknowledgments section. These manuscripts present merely fleeting evidence en route to a next, better, proof; rather than a destination, this is just another starting point.

ABBREVIATIONS

AOM: Acute otitis media
APMGF: Portuguese association of general practitioners
AR: Adrenoceptor
ARDS: Acute respiratory distress syndrome
ASM: Airway smooth muscle
AUC: Area under the curve
BoV: Bocavirus
BPD: Bronchopulmonary dysplasia
CHD: Congenital heart disease
CI: Confidence interval
CLD: Chronic lung disease
COMET: Core outcome measures in effectiveness trials (initiative)
COPD: Chronic obstructive pulmonary disease
COS: Core outcome sets
COSMIN: Consensus-based standards for the selection of health measurement instruments (initiative)
ED: Emergency department
ES: Effect size
FRC: Functional residual capacity
GP: General practitioner
GR: Glucocorticoid receptor
GRADE: Grading of recommendations assessment, development and evaluation (initiative)
GRE: Glucocorticoid-response elements
hMPV: Human metapneumovirus
ICC: Intraclass correlation coefficient
ICD: International classification of diseases
ICU: Intensive care unit
IQR: Interquartile range
ITT: Intention-to-treat
LoA: Limits of agreement
LOS: Length of stay
LRTI: Lower respiratory tract infection
M: Muscarinic (receptor)

MANOVA: Multivariate analysis of variance
MD: mean difference
MIC: Minimal important change
MID: Minimal important difference
NMA: Network meta-analyses
NNT(B)(H): number needed to treat (to benefit)(to harm)
OMERACT: Outcome measures in rheumatology (initiative)
OR: Odds ratio
PCA: Principal component analysis
PEEPi: Positive end-expiratory pressure (intrinsic)
PICU: Pediatric intensive care unit
PIV: Parainfluenza
RACS: Respiratory assessment change score
RCT: Randomized controlled trial
RDAI: Respiratory distress assessment instrument
ROC: Receiver operating characteristic
RR: Risk ratio
RSV: Respiratory syncytial virus
RV: Rhinovirus
SABA: Short-acting β2-adrenoceptor agonists
SatO2: oxygen saturation
SD: Standard deviation
SDC: Smallest detectable change
SEM: Standard error of measurement
SMD: Standardized mean difference
SPP: Portuguese society of pediatrics
UK: United Kingdom
US: United States
WHO: World Health Organization

LIST OF FIGURES

Chapter 4

LIST OF TABLES

SUMMARIES

ABSTRACT (SHORT)/RESUMO (CURTO)

ABSTRACT (LONG)/RESUMO (LONGO)

ABSTRACT (SHORT)

Acute viral bronchiolitis is the most common acute infection of the lower respiratory tract during the first year of life. It is a major cause of clinical morbidity and financial health burden, and encompasses a spectrum of disease severity. This thesis addresses the uncertainties of current evidence on two widely used treatments for bronchiolitis, i.e. bronchodilators and corticosteroids, and how this evidence is limited by shortcomings in key areas of clinical trial design, namely disease definition and outcome selection and measurement. The thesis specific aims were: 1. to assess the comparative efficacy and safety of bronchodilators and corticosteroids, used alone or in combination; 2. to identify outcomes reported in previous clinical trials in bronchiolitis, and a. to assess which outcomes are considered most important to different physicians, and b. to study the measurement properties of two commonly used respiratory distress scales (Respiratory Distress Assessment Instrument - RDAI, and the Respiratory Assessment Change Score - RACS); and 3. to study how physicians define bronchiolitis. **Chapter 1** presents an overview of relevant epidemiological, clinical and pathophysiological findings in bronchiolitis, preceded by a historical perspective. **Chapter 2** describes the results of a comprehensive comparative effectiveness systematic review of bronchodilators and corticosteroids, including 48 trials (4897 patients and 13 comparisons), with network meta-analysis. **Chapter 3.1** presents an exploratory study to identify outcome domains and measurement instruments reported in 90 clinical trials of bronchiolitis included in 11 Cochrane systematic reviews. In **Chapter 3.2**, we report on a measurement study which evaluates the validity, reliability and responsiveness of RDAI and RACS, including data from up to 1765 infants with bronchiolitis enrolled in pediatric emergency departments. Finally, in **Chapter 4** we present results from a nationwide electronic survey of pediatricians and general practitioners, where we assessed physician perspectives on both definition of bronchiolitis, and relevant outcomes and outcome domains for future bronchiolitis trials.

Keywords: bronchiolitis, wheezing, outcomes, measurement, meta-analysis

RESUMO (CURTO)

A bronquiolite aguda é a mais frequente infecção das vias aéreas inferiores durante o primeiro ano de vida, e tem um impacto clínico e económico substancial. Esta tese avalia a evidência actual sobre o uso de broncodilatadores e corticoesteróides, e de que forma essa evidência está limitada por dois aspectos metodológicos chave para o desenho de ensaios clínicos nesta área: a definição de bronquiolite, e a escolha e medição de "outcomes". Os objectivos específicos incluem: 1. avaliar a eficácia e segurança comparativas de broncodilatadores e corticoesteróides, usados isoladamente ou em combinação; 2. identificar os "outcomes" reportados em ensaios clínicos de bronquiolite, e a. avaliar que "outcomes" são considerados mais relevantes por médicos, e b. estudar as propriedades de medida de duas escalas de dificuldade respiratória frequentemente usadas (Respiratory Distress Assessment Instrument - RDAI, e Respiratory Assessment Change Score - RACS); e 3. avaliar perspectivas médicas sobre a definição de bronquiolite. No **Capítulo 1** revemos aspectos epidemiológicos, fisiopatológicos e clínicos da bronquiolite, enquadrados numa perspectiva histórica. No **Capítulo 2** descrevemos os resultados de uma revisão sistemática comparativa sobre a eficácia e segurança de broncodilatadores e corticoesteróides, incluindo 48 ensaios (4897 doentes e 13 comparações), com meta-análise em rede. O **Capítulo 3.1** apresenta um estudo exploratório que identifica domínios de "outcomes" e instrumentos de medida reportados em 90 ensaios clínicos de bronquiolite incluídos em 11 revisões sistemáticas Cochrane. No **Capítulo 3.2** descrevemos um estudo de medição em que se avaliam a validade, fiabilidade e responsividade das escalas RDAI e RACS, incluindo dados de até 1765 crianças no contexto de bronquiolite na urgência pediátrica. Por fim, no **Capítulo 4** apresentamos os resultados de um inquérito electrónico nacional a médicos pediatras e de medicina geral e familiar, avaliando as perspectivas médicas sobre definição de bronquiolite, e sobre quais os "outcomes" considerados relevantes para futuros ensaios clínicos nesta área.

Palavras-chave: bronquiolite, sibilância, outcomes, medição, meta-análise

ABSTRACT (LONG)

Acute viral bronchiolitis is the most common acute infection of the lower respiratory tract during the first year of life. It is a major cause of clinical morbidity and financial health burden, and encompasses a spectrum of disease severity. Seasonal variation in incidence is attributed to the dynamics of viral transmission of its most frequent agent, respiratory syncytial virus. Despite its global impact, our understanding of the immunopathogenesis of this condition remains incomplete. Bronchiolitis is likely the result of a complex interplay between viral agent cytotoxicity, dysregulated host immune response, and environmental factors. The majority of children who develop bronchiolitis are healthy term infants without any known predisposing factors, and most have a mild course. Prognostic factors include demographic, environmental, and family history determinants, as well as comorbidities. A growing body of epidemiological and translational evidence supports an association between bronchiolitis and recurrent wheeze and asthma, likely with complex and bidirectional causal links between these conditions.

This thesis addresses the uncertainties of current evidence on two widely used treatments for bronchiolitis, i.e. bronchodilators and corticosteroids, and how this evidence is limited by shortcomings in key areas of clinical trial design, namely disease definition and outcome selection and measurement. The specific aims were: 1. to assess the comparative efficacy and safety of bronchodilators and corticosteroids, used alone or in combination; 2. to identify outcomes reported in previous clinical trials in bronchiolitis, and a. to assess which outcomes are considered most important to different physicians, and b. to study the measurement properties of two of the most commonly used respiratory distress instruments (the Respiratory Distress Assessment Instrument - RDAI, and the Respiratory Assessment Change Score - RACS); and 3. to study how physicians define bronchiolitis.

Chapter 1 presents an overview of relevant epidemiological, clinical and pathophysiological findings in bronchiolitis, preceded by an historical perspective. It provides a rationale for the main topics addressed in the thesis, and how these can be addressed by recent methodological developments in trial design and synthesis research, including network meta-analysis, core outcome set development, and phenotype-based approaches to the classification of wheezing disorders.

Therapeutic management of bronchiolitis is an ever-controversial topic in pediatrics. There is substantial variation in treatment throughout the world, with most interventions failing to show consistent and relevant treatment effects. Conflicting evidence has emerged regarding the use of two commonly used treatments, bronchodilators and corticosteroids, alone or in combination, as results from the two largest randomised clinical trials in this field were recently published. **Chapter 2** describes the results of a comprehensive comparative effectiveness systematic review of these two treatments, with 48 trials including 4897 patients and 13 comparisons. Evidence from both direct and indirect comparisons was considered, and network meta-analysis performed; a separate Cochrane review focused on corticosteroids is reported. Results do not support a clinically relevant stand-alone effect of systemic or inhaled corticosteroids, β2-adrenergic agonist or anticholinergics on most measured outcomes. Nebulized adrenaline (epinephrine) was beneficial for short term outcomes among outpatients, reducing hospital admissions on day 1 (risk ratio (RR) 0.67, 95% confidence interval (CI) 0.50 to 0.89; number needed to treat (NNT) 15), but not on day 7. Exploratory evidence from a single large trial suggested a longer term synergistic effect of combined treatment with systemic high-dose dexamethasone in outpatients (admissions on day 7 - RR 0.65, 95% CI 0.44 to 0.95; NNT 11). These latter results should be interpreted cautiously due to methodological caveats. While no relevant differences were found in short-term adverse effects for these interventions, harms of combined therapy need to be clarified further. None of the tested interventions were found to be beneficial in hospitalized patients. Overall, both direct and indirect comparisons supported these findings, and network meta-analysis allowed us to rank interventions in outpatients: for admissions on day 1, the probabilities of being the best treatment for adrenaline alone or combined with corticosteroids were 45% and 39%, respectively. Our findings provide greater clarity for clinical decision-making regarding the relative benefits and harms of corticosteroids and bronchodilators in bronchiolitis. They also have implications for the design and conduct of future clinical trials and systematic reviews in this field.

One of the key limitations of this comparative effectiveness review was the heterogeneity in the selection of outcomes and outcome measurements in included bronchiolitis trials. **Chapter 3.1** presents an exploratory study to identify outcome

domains and measurement instruments reported in 90 clinical trials of bronchiolitis included in 11 Cochrane systematic reviews. We classified outcome domains according to two recent conceptual frameworks (by Sinha et al, and by the OMERACT initiative). We found that reported outcome measurements were mostly restricted to short-term clinician-based assessments of clinical severity/respiratory distress (97%) and healthcare use domains (59%), while few measured caregiver-reported symptoms (14%) and quality of life (1%), or long-term outcomes (6%). The same was found for outcomes used to power these trials. Further, 23 different measurement instruments were identified, a majority of which were respiratory distress scales, while a few also encompassed other dimensions of disease severity (e.g. feeding, global status). The most frequently used scales were RDAI and RACS. Timings of measurement, metrics and methods of analysis differed widely. This preliminary work highlights the gaps in measured outcome domains and discrepancies in measurement instruments in bronchiolitis trials.

Limitations in outcome selection could be addressed with the development and application of agreed standardized sets of outcomes ('core outcome sets' - COS), to be measured and reported, as a minimum, in all relevant clinical trials for a specific condition. Initiatives such as OMERACT and COMET have contributed to support the development and implementation of COS. Results presented in **Chapter 4** provide a first contribution to assess physician perspectives on "what to measure" in COS, i.e. relevant outcomes and outcome domains in bronchiolitis trials. We report on a large-scale nationwide online survey (ABBA study) including 514 pediatricians and 165 general practitioners (GPs). The top ranked and rated outcomes by both pediatricians and GPs were hospital admission and respiratory distress. Most outcomes that pediatricians scored above a commonly used threshold for consensus (i.e. 80%) were focused on core areas and domains of health resource use (hospital admission and length of stay), and pathophysiological manifestations, including clinical severity (respiratory distress and need for oxygen therapy), pulmonary function, and disease-related long-term manifestations (recurrent wheezing and asthma). Outcomes relating to life impact, such as quality of life or sleep, were more valued by GPs. Future steps in the development of a COS for bronchiolitis should consider effective methods for engaging, informing and obtaining consensus among key stakeholder groups, particularly parents.

When selecting instruments to measure outcomes from a COS (i.e. "how to measure"), it is imperative that their measurement properties are adequate and applicable for a purpose of evaluation. It is known that many respiratory scales were developed ad hoc, and their measurement properties have not been studied adequately. In **Chapter 3.2**, we provide data on the validity, reliability and responsiveness of RDAI and RACS. We included data from up to 1765 infants with bronchiolitis enrolled in two studies conducted in pediatric emergency departments (ED). We assessed RDAI construct validity by testing hypotheses of associations with physiological measures (respiratory rate, SatO2) and with constructs related to hospitalization, using correlation coefficients and multivariable analysis. RDAI/ RACS responsiveness was evaluated using anchors of change based on these constructs; measures of responsiveness included the area under the curve (AUC). RDAI test-retest agreement and inter-rater reliability were evaluated using limits of agreement (LoA) and Intraclass Correlation Coefficients (ICC). We found that baseline RDAI scores were weakly correlated with respiratory rate ($r=0.38$, $p<0.001$), and scores increased in lower SatO2 categories ($p<0.001$). Higher RDAI scores were associated with hospitalization (odds ratio 1.36, 95% CI 1.26 to 1.47); scores differed between participants that were discharged, admitted or stayed in ED ($p<0.001$). Our hypotheses were met, but the magnitude of associations was below our predefined thresholds. RDAI test-retest LoA were -3.80 – 3.64 (20% of the range), while inter-rater reliability was good (ICC=0.93). Formulated hypotheses for responsiveness were confirmed, with moderate responsiveness (AUC: RDAI 0.64 – 0.70; RACS 0.72). We concluded that RDAI has poor to moderate construct validity, with good discriminative properties but considerable test-retest measurement error. RDAI and RACS are responsive measures of respiratory distress in bronchiolitis, but do not encompass all determinants of disease severity. These results suggest that both scales have limitations in their use as evaluative trial outcome measures.

While bronchiolitis is a relatively straightforward clinical diagnosis for most child health practitioners, no standardised set of diagnostic criteria exists. The label 'bronchiolitis' may overlap with acute wheezing and asthma, which hampers the interpretation of current evidence. In **Chapter 4**, we present results from the ABBA study focusing on perspectives of paediatricians and GPs on definition of bronchiolitis. We used principal component analysis (PCA) to explore dimensions underlying disease definition. Most paediatricians (76.5%) agreed with a definition

based on coryza, wheezing and/or crackles/rales, compared to 38.1% GPs ($\chi 2$, $p<0.001$). Less than 5% physicians agreed with a definition commonly used in clinical trials (<12 months, first episode of wheeze). We retained three dimensions on PCA: one based on coryza, rales/crepitations and no sudden onset; another on number of episodes and age; and a third on wheeze. Dimensions varied by physician specialization and training ($p<0.01$). Thus, physician definitions of bronchiolitis have considerable variability and often mismatch those of clinical trials. These results highlight the need for a robust standardised definition of acute bronchiolitis.

RESUMO (LONGO)

A bronquiolite aguda é a infecção das vias aéreas inferiores mais frequente durante o primeiro ano de vida. Tem um impacto clínico e económico substancial, e engloba um largo espectro de gravidade clínica. Apesar do seu impacto global, a imunopatogénese da doença permanece pouco esclarecida, resultando de uma combinação complexa de citotoxicidade viral, resposta imunitária desregulada, e factores ambientais. Entre os factores prognósticos contam-se determinantes demográficos, ambientais e familiares, e co-morbilidades. Existe crescente evidência de uma associação causal complexa e bidirecional entre bronquiolite, sibilância recorrente e asma.

Esta tese avalia a evidência terapêutica actual em bronquiolite, e de que forma essa evidência está limitada por dois aspectos metodológicos chave para o desenho de ensaios clínicos nesta área: a definição de bronquiolite, e a escolha e medição de "outcomes". Os objectivos específicos incluem: 1. avaliar a eficácia e segurança comparativas de broncodilatadores e corticoesteróides, usados isoladamente ou em combinação; 2. identificar os "outcomes" reportados em ensaios clínicos, avaliar que "outcomes" são considerados mais relevantes por médicos, e estudar as propriedades de medida de duas escalas frequentemente usadas (Respiratory Distress Assessment Instrument - RDAI, e Respiratory Assessment Change Score - RACS); e 3. avaliar perspectivas médicas sobre a definição de bronquiolite.

No **Capítulo 1** revemos aspectos epidemiológicos, fisiopatológicos e clínicos da bronquiolite, enquadrados numa perspectiva histórica. Apresentamos os fundamentos que suportam esta tese, e descrevemos desenvolvimentos metodológicos no desenho de ensaios clínicos e de revisões sistemáticas e meta-análises, incluindo a meta-análise em rede, os *core outcome sets* (COS) (i.e. conjuntos de "outcomes" essenciais), e a identificação de fenótipos em doenças respiratórias com sibilância.

A abordagem terapêutica da bronquiolite é um tema historicamente controverso. Há variabilidade nas práticas a nível global, e a evidência é contraditória quanto ao uso de broncodilatadores e corticoesteróides. No **Capítulo 2** descrevemos os resultados de uma revisão sistemática comparativa sobre a eficácia e segurança destes dois fármacos, incluindo 48 ensaios (4897 doentes e 13 comparações).

Utilizámos evidência de comparações directas e indirectas, e efectuámos meta-análise em rede; uma revisão Cochrane sobre corticoesteróides é descrita separadamente. O uso isolado de corticoesteróides inalados ou sistémicos, agonistas β2-adrenérgicos, e anticolinérgicos, não se associou a um efeito terapêutico clinicamente relevante para a maioria dos "outcomes". O uso de adrenalina (epinefrina) nebulizada foi benéfico para "outcomes" a curto prazo em urgência hospitalar, com redução dos internamentos ao 1º dia (risco relativo (RR) 0.67, intervalo de confiança 95% (IC95%) 0.50 a 0.89; número necessário tratar (NNT) 15), mas não ao 7º dia. Evidência exploratória de um ensaio clínico sugere um efeito sinérgico prolongado ao combinar adrenalina com dexametasona sistémica em dose alta (internamentos ao 7º dia - RR 0.65, IC95% 0.44 a 0.95; NNT 11). Porém, estes resultados devem ser interpretados com precaução, face a questões metodológicas. Não houve diferenças relevantes nos efeitos adversos a curto prazo, mas a segurança desta terapêutica combinada deve ser clarificada. Nenhuma das intervenções mostrou benefício em crianças internadas. Os resultados de comparações directas e indirectas foram consistentes, e a meta-análise em rede classificou as intervenções pela probabilidade de ser o melhor tratamento (45% para a adrenalina isolada e 39% para a terapêutica combinada para internamentos ao 1º dia). Estes resultados clarificam os benefícios e riscos comparativos do uso de corticoesteróides e broncodilatadores, e permitem emitir recomendações para futuros ensaios clínicos e revisões sistemáticas nesta área.

Uma das limitações identificadas por esta revisão sistemática foi a heterogeneidade na selecção de "outcomes" e instrumentos de medida. O **Capítulo 3.1** apresenta um estudo exploratório que identifica domínios de "outcomes" e instrumentos de medida reportados em 90 ensaios clínicos de bronquiolite incluídos em 11 revisões sistemáticas Cochrane. Analisámos os domínios de "outcomes" de acordo com duas classificações conceptuais recentes (por Sinha et al, e pela iniciativa OMERACT). Constatámos que a maioria dos "outcomes" reportados estava restrita a avaliações clínicas da gravidade e dificuldade respiratória efectuadas por profissionais de saúde e medidas a curto prazo (97%), ou a domínios de uso de cuidados de saúde (59%). Pelo contrário, poucos "outcomes" avaliavam as perspectivas dos cuidadores sobre os sintomas (14%) ou qualidade vida (1%), assim como "outcomes" a longo prazo (6%). Por outro lado, identificámos 23 instrumentos de medida diferentes, a maioria dos quais escalas de dificuldade

respiratória. Um pequeno numero de escalas incluía igualmente outras dimensões da gravidade da doença (e.g. estado geral, nutrição). As escalas mais frequentemente utilizadas foram a RDAI e a RACS. Constatou-se igualmente variabilidade no tempo de medição, na métrica usada e nos métodos de análise. Este estudo realça as lacunas na selecção de "outcomes" e as discrepâncias nos instrumentos de medida usados em ensaios clínicos de bronquiolite.

Estas limitações poderiam ser ultrapassadas através do desenvolvimento e aplicação de COS em todos os ensaios clínicos, tal como proposto pelas iniciativas OMERACT e COMET. No **Capítulo 4** descrevemos um primeiro contributo para avaliar as perspectivas de médicos sobre "o que medir" nestes COS, i.e. "outcomes" e domínios de "outcomes" relevantes. Trata-se de um inquérito electrónico nacional (estudo ABBA) que incluiu 514 pediatras e 165 médicos de medicina geral e familiar (MGFs). Os "outcomes" mais pontuados e melhor classificados por ambos os grupos foram o internamento hospitalar e a dificuldade respiratória. A maioria dos "outcomes" que os pediatras pontuaram acima de um limiar usado para consenso (80%) focavam domínios de uso de recursos de saúde (internamento hospitalar e duração de internamento), e manifestações fisiopatológicas, incluindo gravidade clínica (dificuldade respiratória e necessidade de oxigenioterapia), função pulmonar, e manifestações a longo prazo (sibilância recorrente e asma). Os MGFs valorizaram mais "outcomes" relacionados com impacto na via diária, como qualidade de vida ou sono. Um futuro COS em bronquiolite deve também envolver, informar e obter consenso com outros grupos de interesse, em particular pais e cuidadores.

A escolha de instrumentos para medir "outcomes" num COS (i.e. "como medir") implica que as suas propriedades de medição sejam adequadas, mas estas não estão adequadamente estudadas para muitas escalas respiratórias. No **Capítulo 3.2** descrevemos um estudo de medição em que se avaliam a validade, fiabilidade e responsividade das escalas RDAI e RACS. Incluímos dados de até 1765 crianças com bronquiolite, recrutadas em dois estudos que decorreram no contexto de urgência pediátrica. Avaliámos a validade de constructo da RDAI pela formulação de hipóteses sobre a associação das pontuações da escala com medidas fisiológicas (frequência respiratória, SatO2) e com constructos relacionados com o internamento, usando coeficientes de correlação e análise multivariada. A

responsividade da RDAI e da RACS foi avaliada recorrendo a referenciais de mudança baseados nos constructos referidos; entre as medidas de responsividade incluíram-se a area sob a curva (AUC). A concordância teste-reteste da RDAI e a fiabilidade inter-observador foi estudada usando os limites da concordância (LoA) e os coeficientes de correlação intra-classe (ICC). Constatámos que as pontuações RDAI estavam fracamente correlacionadas com a frequência respiratória ($r=0.38$, $p<0.001$), e que as pontuações aumentavam para categorias de SatO2 inferiores ($p<0.001$). Pontuações de RDAI superiores associaram-se a maior risco de internamento hospitalar (odds ratio 1.36, IC95% 1.26 a 1.47); houve diferenças significativas entre participantes que foram internados, que se mantiveram na urgência ou que tiveram alta ($p<0.001$). Embora as hipóteses formuladas se tenham confirmado, a magnitude das associações não atingiu os limiares que pré-definimos. Os LoA para a concordância teste-reteste da RDAI foram -3.80 – 3.64 (20% da amplitude da escala), e a fiabilidade inter-observador foi boa (ICC=0.93). Confirmámos as hipóteses formuladas para avaliar a responsividade, que foi moderada (AUC: RDAI 0.64 – 0.70; RACS 0.72). Concluímos que a RDAI tem validade de constructo pobre a moderada, com boas propriedades discriminativas mas considerável erro de medição. A RDAI e a RACS são medidas responsivas de dificuldade respiratória na bronquiolite, mas não abrangem todos os determinantes da gravidade da doença, o que limita o seu uso na prática clínica e na investigação.

Embora o diagnóstico de bronquiolite seja habitualmente simples para os profissionais de saúde em pediatria, não existem critérios diagnósticos estandardizados. O rótulo 'bronquiolite' pode sobrepor-se aos diagnósticos de sibilância aguda e asma, o que limita a interpretação da evidência terapêutica. No **Capítulo 4** apresentamos os resultados do estudo ABBA referentes às perspectivas de pediatras e MGFs sobre definição de bronquiolite. Utilizámos análise de componentes principais (PCA) para explorar as dimensões subjacentes à definição de bronquiolite. A maioria dos pediatras (76.5%) concordaram com uma definição baseada na presença de coriza, sibilância e/ou fervores/roncos, em comparação com 38.1% MGFs ($p<0.001$). Menos de 5% dos médicos concordou com uma definição habitualmente usada em ensaios clínicos (<12 meses, primeiro episódio de sibilância). Retivemos três dimensões através de PCA: uma baseada na presença de coriza e de fervores/crepitações, e ausência de início súbito; outra baseada no número de episódios e na idade; e outra na presença de sibilância. As dimensões

variaram consoante a especialidade médica e os anos de formação ($p<0.01$). Em conclusão, constatámos heterogeneidade nas definições de bronquiolite, frequentemente divergentes das utilizadas em ensaios clínicos, o que realça a necessidade de uma definição robusta e estandardizada.

LIST OF PUBLICATIONS/ABSTRACTS

Published

Hartling L, Fernandes RM, Bialy L, Milne A, Johnson D, Plint A, Klassen TP, Vandermeer B. Steroids and bronchodilators for acute bronchiolitis in the first two years of life: systematic review and meta-analysis. British Medical Journal, 2011;342:d1714.

Fernandes RM, Bialy LM, Vandermeer B, Tjosvold L, Plint AC, Patel H, Johnson DW, Klassen TP, Hartling L. Glucocorticoids for acute viral bronchiolitis in infants and young children. Cochrane Database of Systematic Reviews 2010, Issue 10. Art. No.: CD004878. DOI: 10.1002/14651858.CD004878.pub3.

Fernandes RM, Bialy LM, Vandermeer B, Tjosvold L, Plint AC, Patel H, Johnson DW, Klassen TP, Hartling L. Glucocorticoids for acute viral bronchiolitis in infants and young children. Cochrane Database of Systematic Reviews 2013, Issue 6. Art. No.: CD004878. DOI: 10.1002/14651858.CD004878.pub4.

Fernandes RM, Hartling L. Glucocorticoids for acute viral bronchiolitis in infants and young children. Journal of the American Medical Association. 2014;311:87-8.

Fernandes RM, Plint AC, Terwee CB, Sampaio C, Klassen TP, Offringa M, van der Lee JH. Validity of Bronchiolitis Outcome Measures. Pediatrics, in press.

Abstracts / manuscripts submitted and under review

Fernandes RM, Andrade GM, Constant C, Malveiro D, Magalhães M, Abreu D, Azevedo I, Sousa E, Salgado R, Bandeira T. Perspectives of pediatricians and general practitioners on definition and important outcomes in bronchiolitis. Eur Respir J 2014 44:Suppl 58, P1257 / submitted and under review by Pediatric Pulmonology.

Fernandes R. Bronchiolitis core outcome set. Third meeting of the COMET (Core Outcome Measures in Effectiveness Trials) Initiative, Liverpool, 20 June 2013.

CHAPTER 1

GENERAL INTRODUCTION

1.1 An Overview Of Bronchiolitis

1.2 The Dilemmas And Limitations Of Intervention Research In Bronchiolitis

1.3 Objectives And Outline Of The Thesis

Acute viral bronchiolitis is the most common acute infection of the lower respiratory tract during the first year of life.[1] It is a major cause of clinical morbidity and its financial burden is substantial. The diagnosis is clinical in infants and young children, based on a history of rhinorrhoea and low-grade fever that progress to cough and respiratory distress, with findings of tachypnoea, chest retractions and wheeze, crackles, or both, on examination.[2,3] Although bronchiolitis is usually a straightforward diagnosis common to all pediatricians, some variability in its definition exists.[4]

Respiratory syncytial virus (RSV) is responsible for the majority of cases, usually in seasonal epidemics, but other viral agents may also be involved as single or dual infections.[5] Bronchiolitis is characterized by acute bronchiolar inflammation and submucosal edema, impaired mucociliary clearance, necrosis of small airway epithelial cells and increased mucus production.[6] Disease severity is likely the result of a complex interplay between host, agent and environmental factors.[6] Further, basic, translational and clinical research studies are elucidating the links between bronchiolitis, preschool wheezing disorders and later asthma and long-term respiratory morbidity.[7] Treatment of bronchiolitis is an ever-controversial topic. There is substantial variation in its management throughout the world, and claims of efficacy of many interventions have been challenged by the growing evidence base from recent large randomized controlled trials.[8-10]

In this introductory chapter we present an overview of relevant epidemiological, clinical and pathophysiological findings in bronchiolitis, preceded by an historical perspective of milestones in research in this field. These provide a background for the interpretation of current of evidence regarding treatment interventions, such as corticosteroids and bronchodilators. We then identify several shortcomings in current evidence in this field, and how recent methodological developments in clinical trial design and synthesis research may help address them. We end by presenting the objectives of this thesis, and outlining its structure.

1.1 AN OVERVIEW OF BRONCHIOLITIS

The sources cited in this chapter were selected by searching PubMed and the Cochrane database in mid-2014 using the term "bronchiolitis" and associated terms for each topic addressed. We hand selected what we deemed to be scientific and clinically relevant articles, with preference given to systematic reviews with explicit quality assessment criteria, high-quality randomized and observational studies, and key translational research. We also drew on our personal archive of references.

HISTORICAL PERSPECTIVE

The term 'bronchiolitis' is referenced in the medical literature from late 19th century onwards, although it may have been used previously in clinical practice.[11,12] 'Capillary bronchitis' or 'capillary bronchiolitis' were used to describe an inflammatory illness of the terminal bronchioles, based on clinicopathologic correlations done on postmortem studies.[11,13] For years there was controversy as to whether bronchiolitis existed as a pathologic entity, distinct from conditions such as bronchopneumonia and tracheobronchitis. Some authors emphasized that there were no clear boundaries containing the bronchiolar inflammatory process from contiguous spaces, i.e. the tracheobronchial tree and the lobular structure.[12] Capillary bronchiolitis would therefore be either an extension of tracheobronchitis, or an early stage of bronchopneumonia, and might not have relevance per se. However, these early descriptions were mostly based on bronchiolar disease occurring in adults after epidemics of measles in U.S. Army camps during the first World War, or accompanying the influenza pandemic of 1918.[14] This isolated acute condition was otherwise considered rare and was mostly restricted to the extremes of ages, particularly infants and the elderly.[12]

By the early 20th century, authors in the field of pediatrics were reportedly aware of a clinical entity occurring frequently and almost exclusively in young children, characterized by severe respiratory distress and cyanosis.[14] However, reports were sparse and based in disparate cases. The first comprehensive description is unanimously attributed to Hubble et al in a 1941 British Medical Journal paper which described an epidemic of bronchiolitis involving hospitalized children.[15]

That same year, Adams described an outbreak of nosocomial neonatal chest infections, with cytoplasmic inclusions identified in the lungs at autopsy.[16] This was followed by numerous other consistent case series of children with similar clinical findings of obstructive dyspnoea, clinically distinct from classic pneumonia or bronchitis, with a putative role for bronchiolar obstruction.[17,18] However, the discussions on the uniqueness of pathologic findings pervaded, and this "new" condition was initially considered a small portion of obstructive or interstitial pneumonias in traditional pediatric textbooks.[19] Only by the end of 1960s would acute bronchiolitis be finally listed as a distinct clinical entity in reference's such as Nelson's or Holt's Pediatrics.[19] Earlier, however, Engel and Newns had elegantly demonstrated that inflammatory changes in infant bronchiolitis were dominated by bronchiolar findings, and were different from those previously described in adult studies.[13] While the etymology of the word bronchiolitis suggested specific pathologic findings, the label soon started to be applied on the basis of clinical findings alone.

Clarity on the etiology of the syndrome was lagging, however, which further limited its widespread recognition and study. The role of known bacterial and viral agents was disputed, as few were sporadically isolated and identified.[15,18] A major breakthrough occurred between 1956 and 1957, when Chanock and colleagues isolated a novel virus from infants with severe lower respiratory illness.[20,21] The virus had been first isolated from a chimpanzee and was originally called the "chimpanzee coryza virus", but was renamed respiratory syncytial virus (RSV) because of its predilection for the respiratory system and its tendency to produce syncytia when inoculated into human cell lines.[21,22] It was soon found to be responsible for a majority of cases of infants with the clinical complex labelled bronchiolitis seen in several outbreaks during the 1960s.[23-26] These outbreaks enabled a consistent description of distinctive clinical features such as dyspnoea with expiratory wheezing, a disparity between seriousness of symptoms and temperature, and a relative paucity of radiographic findings, with a spectrum of severity ranging from mild disease to deadly cases.

During the following years, relevant developments and failures in different fields provided insight into the pathogenesis, natural history and treatment response of bronchiolitis. The first therapeutic clinical trials started in the late 1960s, testing

various corticosteroids and adrenergic agonists based on a putative role for inflammation and bronchospasm, to mixed results.[27,28] Development of a formalin-inactivated vaccine against RSV was curtailed, as immunized children exposed to RSV in the community and seronegative for the virus before vaccination developed non-protective antibody responses and experienced an increase in the severity of lung disease.[29] Further, links between bronchiolitis, other wheezing disorders and asthma were first suggested by Reynolds and Cook in 1963, who distinguished between bronchiolitis patients in whom airways obstruction was largely attributable to edema of the airways and secretions, and those with a predisposition to asthma.[30] But while our understanding of childhood acute and chronic wheezing disorders and asthma evolved with landmark respiratory birth cohorts such as the Tucson Children's Respiratory Study in the 1980s, that of bronchiolitis as a distinct entity lagged.[1]

The last decades showed a resurgence of interest in bronchiolitis research. The development of palivizumab, the first licensed monoclonal antibody for an infectious disease, was accompanied by many studies on populations at risk of severe RSV bronchiolitis.[31] The role of RSV and other bronchiolitis-related viruses in recurrent wheezing and asthma has recently emerged.[7] Much effort has gone into establishing whether infants who experience severe disease and sequelae do so because of features intrinsic to the agent's virulence, as opposed to characteristics related to the host's own immunological response.[6] The first genetic association and high-throughput genomic expression profile studies open new avenues in the identification of predictors of susceptibility and/or disease progression.[32] Further, bronchiolitis is now the focus of promising drug and biologic development efforts, including targeted therapies and new vaccines.[33-35]

But for clinicians and parents on the front line during every bronchiolitis season, these findings are yet to translate into major breakthroughs in infant care. For more than 50 years the mainstays of treatment have remained oxygen, fluids and, if necessary, respiratory support.[30,36] Oximetry and non-invasive ventilation are two examples of significant improvements in management, but the burden of hospitalizations has been increasing in developed countries, while the impact of disease in developing countries is tremendous.[37-41] While implementation of the first evidence-based guidelines can be traced to the 1990s,[42,43] practice variation

remains considerable.[8,44] Further, pivotal randomized clinical trials (RCTs) for many frequently used interventions are only recent.[45,46] Many controversies on definition and management of bronchiolitis have lasted, partly because of heterogeneity and shortcomings in current studies in this field.

EPIDEMIOLOGY AND BURDEN OF DISEASE

Acute viral bronchiolitis encompasses a spectrum of severity, from mild disease cared for in the community, through cases that require acute care in the emergency department (ED), a proportion of which are hospitalized and may progress to severe disease requiring intensive care, and rarely lead to death. While it is known to be the most common acute infection of the lower respiratory tract during the first year of life, there are remarkably few population-based epidemiological studies that measure incidence and burden of illness across these different severities and settings.[47] Further, a considerable proportion of children have postbronchiolitis symptoms, and the risk of recurrent wheezing and possibly asthma is increased.[48,49] Figure 1.1 aggregates results from selected studies that will be described in this section, in order to provide an overall perspective of the epidemiological impact of bronchiolitis.

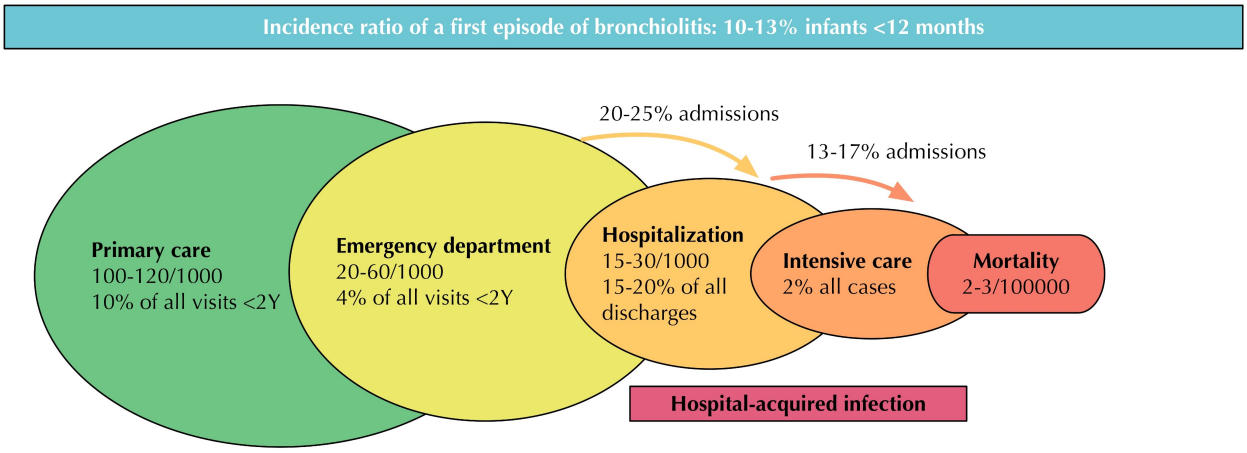


Figure 1.1. Epidemiology of bronchiolitis across settings (based on references presented in the text)

It should be noted that heterogeneity in epidemiological study design and methods contribute to considerable variability in some measures of burden of disease. The following methodological factors can affect estimates: choice of population (e.g. all newborns or selected children at risk such as preterm), operational case definition of bronchiolitis (e.g. age range, specific viral agent), disease severity and setting (e.g. clinic-based or hospital-based), method of case ascertainment (e.g. active prospective surveillance or passive use of administrative health records), and source of denominator of children at risk (e.g. census or population-based). Additionally, differences in how provision of acute care is structured likely impact these measures, for example give heterogeneity in ED and intensive care unit (ICU) admission practices.

Incidence

The Tucson Children's Respiratory Study in the United States (US) was the one the first population-based studies conducted in a developed country that used prospective surveillance to assess the incidence of acute lower respiratory tract infections (LRTI) in infancy, including bronchiolitis.[1,50] While many other respiratory birth cohorts have been conducted, their focus is mostly on recurrent wheezing and asthma, and bronchiolitis per se is rarely an a priori defined measured event or outcome.[51,52] The Tucson cohort of 1149 infants showed a bronchiolitis incidence ratio of approximately 10% within the first year of life.[1] This incidence is comparable with that observed in administrative data studies using International Classification of Diseases (ICD) coding, such as that from Koehoorn et al in 2008, showing 13.4% of singleton infants born in the Georgia Air Basin region in Canada had incident bronchiolitis requiring a clinical encounter in the first 12 months of life.[47] The impact across different levels of care is reflected in another population-based, retrospective, cohort study of about 100000 term, non–low birthweight, otherwise-healthy infants who enrolled in the Tennessee Medicaid Program, from 1995 to 2003, and were followed for one year.[53] Rates of health care visits for treatment of bronchiolitis during infancy were substantial, with about 20% infants having health care visits attributable to bronchiolitis during infancy, 13.3% having an outpatient visit, 6.2% an ED visit, and 5.5% being hospitalized (more than one episode could be included).

Data on incidence is also available from studies focusing on RSV LRTI, the main etiological agent of bronchiolitis. Many of these studies are hospital-based, focusing on episodes of severe RSV-associated LRTI necessitating hospital admission, and are likely to yield falsely low estimates of population incidence. Albeit RSV-based data encompasses other LRTI manifestations such as pneumonia and thus misclassification is possible, there is clinical overlap and known variability in labeling between both diagnoses.[54,55] Community-based studies with active prospective surveillance are rare. The Houston Family Study, a small longitudinal study of RSV infection in children followed up from birth performed in the 1980s, was paramount in assessing the natural history of RSV infection and re-infection.[56] The incidence ratio of primary RSV LRTIs in the first year of life was 21.6%, 81% of which had a diagnosis of bronchiolitis. In a recent birth cohort of healthy newborns, 42 out of 298 (14%) developed RSV LRTI during their first year of life.[57] Studies in different settings confirm the impact of RSV-disease across the spectrum of severity. Hall et al performed a prospective, population-based surveillance of acute respiratory infections in three US counties, enrolling hospitalized children or children presenting as outpatients in EDs and pediatric offices.[54] Among 5067 children enrolled in the study, 919 (18%) had RSV infections. Overall, RSV was associated with 20% of hospitalizations, 18% of ED visits, and 15% of office visits for acute respiratory infections from November through April.

Mortality

Mortality due to bronchiolitis is very low in developed countries, with most estimates around 2 to 3 cases per 100000 live births in the US, United Kingdom (UK) and France.[58-60] Estimates of RSV-related LRTI mortality are comparable.[61] Studies that examined temporal trends in bronchiolitis-associated outcomes have reported either a significant decline or no change in mortality between the 1980s and 2000s.[58,62] The odds of in-hospital mortality from bronchiolitis declined significantly during the last decade in the US.[60]

On the contrary, the impact of bronchiolitis and RSV-disease in developing countries is tremendous. It has been estimated that, globally, between 66000 to 199000 children younger than five years die from RSV-associated LRTI, with 99% of these deaths occurring in developing countries.[38] Additionally, case fatality ratios in

children younger than one year admitted to hospital for RSV-associated severe LRTI are much higher in developing countries.[38]

Hospitalizations, intensive care and hospital-acquired infection

Bronchiolitis is the leading cause of hospitalization in infants. A landmark US study by Shay and colleagues in 1999 found bronchiolitis to be associated with close to 47% of all LRTI discharges and 16% of total discharges in children younger than one year.[37] Most studies in developed countries have shown population-based hospitalizations rates up to 3% or higher within the first year of life, including data from the US,[37,53,54,60,63] Canada,[47,64] England,[65] Sweden,[66] the Netherlands,[67] and France.[59] Many studies have focused on the subset of RSV-bronchiolitis hospitalizations. In a recent systematic review and meta-analysis aimed at studying the global burden of RSV infection, the pooled incidence in developed and developing countries was 5.5 (4.2–7.2) and 5.6 (4.3–7.4) RSV-positive hospitalized cases per 1000 per year in children aged less than a year, respectively.[38] These estimates were highly variable within countries or regions and between regions, which is likely due to the aforementioned methodological factors, as well as differences in sensitivity and specificity of diagnostic assays to identify RSV infection.

Differing trends in bronchiolitis hospitalizations have been found in the last decades. Population studies from the US reported increases through the 1990s and early 2000s that were consistent over time and geographically.[37,53] This increase was also seen in other countries.[64,66,67] By contrast, a recent study in the US found a 17% decrease in the incidence of bronchiolitis hospitalizations nationally between 2000 and 2009, with recent figures at 18.1% of all hospitalizations for children aged less than one year and 19.2 per 1000 person-years.[60] The reasons for these fluctuations in trends are unclear. Variations in hospitalization rates could be due to health-care system differences (e.g. organization, resource allocation, and provision of care), physician practices, or agent/host biological or environmental factors. Explanatory hypotheses for the initial upward trend included an increase in populations at risk such as preterms, and the generalized use of oximetry.[37] However, several studies did not find similar increases in other respiratory illnesses in which pulse oximetry is used routinely.[37] The decrease in hospitalizations could likely be related to changes in child care practice or altered criteria for

hospitalizations.[60] It should be noted that accompanying trends in hospitalization rates for pneumonia and asthma do not support the hypothesis that changes in bronchiolitis admissions are due to diagnostic coding variations.[37,60]

A considerable proportion of hospitalized children with bronchiolitis require intensive care, with an important burden for pediatric intensive care units (PICUs) during seasonal outbreaks. In a large cohort of hospitalized children in the US, 17% were enrolled in the ICU, either directly from the ED or during the course of hospitalization.[68] In a nationwide administrative data study in France, this proportion was about 13%.[59] Between 2000 and 2009, the rate of children with bronchiolitis who required respiratory support (invasive or non-invasive) in the US increased significantly from 1.9% in 2000 to 2.3% in 2009.[60]

As a major cause of hospital admissions, bronchiolitis and RSV LRTI leads to significant inpatient health care costs.[69,70] Curiously, the recent apparent decrease in the incidence of hospitalization and in-hospital mortality in the US contrasts with an increase in national hospital charges.[60]

There is a paucity of published data on the epidemiology of hospital-acquired bronchiolitis infection. RSV has been identified as a nosocomial hazard in pediatric wards, particularly in young children.[71,72] Nosocomial RSV infection may occur during or outside community outbreaks, but reported incidence and transmission rates vary widely.[73-76] Outbreaks of nosocomial transmission in pediatric and neonatal ICUs may cause considerable impact, and children with co-morbidities or who are technology dependent are at increased risk.[77,78] Hospital-acquired RSV bronchiolitis may also increase risk of hospital readmission.[79] Overall, the risk and cost of nosocomial RSV infection contributes to the overall burden of RSV.[80]

Outpatient care

While data on inpatients has been accumulating, the interest in the impact of bronchiolitis in community and outpatient settings is relatively recent. Yet results from aforementioned population-based studies highlight how hospitalizations are a tip of the iceberg of bronchiolitis burden of disease, with up to four times the number of outpatient visits.[53] Mansbach et al have studied extensively the large impact of bronchiolitis as a motive for ED and office visits in the US throughout the

last decade, with reported rates around 35 ED visits per 1000 person-years, accounting for about 4% of all ED visits below two years of age, with higher rates (up to 60 per 1000) below six months.[81-83] Large scale data is also available from Alberta, Canada, with standardized rates of about 40 ED visits per 1000 in 2004/2005.[84] Further, comparative data from Bourgeois et al based on three patient cohorts show how, in children, significantly more ED visits can be attributed to RSV as opposed to influenza.[85]

The overall hospital admission rates vary between 20 to 25%.[81,83,84,86] Admission rates from the ED are known to vary by organizational factors such as type of ED (general vs pediatric).[86] It must be noted that most available data is from North American centers, and differences in acute care health services and patient management will likely lead to regional variation in these measures.

Temporal trends for outpatient care have not necessarily followed those of hospitalizations. US ED visit rates for bronchiolitis were stable between 1992 and 2000, and there were increasing trends in the early 2000s within a local population and patients with RSV only, and similar trends were found in Canada.[53,54,81,84] A recent update looking at US data between 2006 and 2010 found a divergent temporal trend by age group, with an increase among children from 12 months to 23 months, and a significant decline below that age.[83]

Although relatively low at the individual level, the costs for ED expenses of bronchiolitis are considerable given the incidence of disease.[87] This adds to the economical impact in the community through outpatient visits and loss of parental work time.[69,82,88]

AGENT, HOST AND BRONCHIOLITIS SEVERITY: A COMPLEX INTERPLAY

Agents

RSV is the most common pathogen associated with bronchiolitis, but other relevant viruses include rhinovirus (RV), human metapneumovirus (hMPV), influenza A/B, parainfluenza (PIV), and adenovirus.[56,89-95] Another recently discovered parvovirus,

human bocavirus (BoV), has also been linked to bronchiolitis.[96-98] The clinical relevance of more recently identified viruses such as novel polyomaviruses and coronaviruses is uncertain.[99-105] There is conflicting literature about the relevance of bacterial co-infection in children with bronchiolitis, which may be relevant in children requiring intensive care.[106-110]

Respiratory viruses differ in structure and properties, but are known to be associated with a variety of acute upper and lower respiratory conditions, across age groups and co-morbidities. RSV is an enveloped Pneumovirus of the Paramyxoviridae family containing a negative-sense, single-stranded RNA genome.[6] There are two major antigenic subgroups, A and B, which are defined by different envelope proteins and co-circulate each year. While usually associated with morbidity in infants and young children, RSV also carries substantial burden in elderly and high-risk adults.[111] hMPV is a RNA virus discovered in 2001 that belongs to the same family and genus of RSV.[112] Human RVs, members of the family Picornaviridae, were first identified in culture in 1956, and currently, more than 100 serotypes have been described.[112] Human RV serotypes were first classified into 2 phylogenetic groups, group A and group B, but recently, a novel group group C has been isolated.[113,114] Although once thought to cause only common cold, it is now known that RVs are associated with LRTI, asthma exacerbations, exacerbations of chronic lung disease, sinusitis, and otitis media.[114-116] Adenoviruses are double-stranded DNA viruses belonging to the family Adenoviridae. There are at least 51 known serotypes of adenovirus, which are categorized into six subgenera (A to F), associated with both respiratory and enteric infections.[112] Influenza virus is a negative-sense single-stranded RNA virus belonging to the family Orthomyxoviridae, responsible for flu epidemics and pandemics, while PIV is a negative-sense single-stranded RNA virus belonging to the family Paramyxoviridae, with four serotypes responsible for a spectrum of respiratory tract infections, particularly croup.[112]

Observational studies have examined the epidemiology of different viruses associated with bronchiolitis in different settings, but some methodological caveats must be considered when interpreting results. First, many studies do not focus specifically on bronchiolitis, but also include acute recurrent wheezing episodes and other LRTI manifestations. Age ranges and co-morbidities of included children

may also differ. Second, while most studies have been performed in hospitalized children, less evidence is available from children with less severe disease in outpatient care. Further, the diagnostic accuracy of viral detection methods is variable and has evolved through the last decades. The first descriptive studies of the viral etiology of bronchiolitis in the 1960 through the 1980s primarily used traditional diagnostic methods such as cell culture, antigen detection, and serologic testing. New molecular diagnostic technology, especially multiplex polymerase chain reaction, greatly improved the detection of known viruses, allowing the identification of apparently "new" viruses, and highlighting the existence of co-infection.[112] However, these improvements in viral detection do not prove that there is a pathological role for these viruses. There are numerous challenges in proving viruses as the etiologic causes of specific syndromes such as bronchiolitis.[117,118] These include, among others, the interpretation of asymptomatic viral shedding in children (e.g. frequent in RV or BoV), or the significance of detecting agents using different specimen collection techniques at different locations in the respiratory tract (e.g. nasopharyngeal aspirates vs bronchoalveolar lavage).[119-121] Lastly, better analytical validity and diagnostic accuracy in detection viruses do not necessarily translate into clinical utility and improvements in patient management.[122]

The epidemiology of bronchiolitis viral agents at different levels of severity and settings can be illustrated by three recent major studies performed at distinct levels of care, i.e. inpatient, ED, outpatient clinic. All studies used molecular viral detection methods and had a high proportion of viral isolation. The Multicenter Airway Research Collaboration of the US Emergency Medicine Network conducted a prospective, multicenter cohort study of hospitalized children younger than two years during the 2007-2010 winter seasons.[5] Of 2207 participants, 1410 children (64%) had a single virus infection and 658 (30%) had two or more viruses; the remaining 139 children (6%) had no pathogen identified from an extended molecular testing panel. The most common agents were RSV (72%) and RV (26%); the incidence of each of the other viruses were 8% or less. Co-infections were found in 32% of children who tested positive for RSV, 23% of those who had negative test results for RSV, and mostly 70% of children with RV. These frequencies are comparable to older studies performed in Europe by Jartti and colleagues, and Calvo and colleagues, albeit these studies had slightly more cases of non-RSV non-RV viruses isolated.[95,123] Results in outpatients show similarities but also some

discrepancies. In a prospective, multicenter cohort study of 277 children aged less than two years presenting to US EDs with physician-diagnosed bronchiolitis, Mansbach et al examined the frequencies of RSV, RV, hMPV, and influenza A/B using nasopharyngeal aspirates collected during one bronchiolitis winter season.[124] At least a virus was detected in 84% of the samples, and multiple pathogens were identified in 9%. All cases considered, the two most common agents were RSV (64%) and RV (16%), and the most common co-infection was RSV with RV. Kusel et al performed a landmark community-based birth cohort study of 263 Australian children aged less than 12 months, at high risk for atopy.[119] All acute respiratory infections, including bronchiolitis, were prospectively surveilled, and nasopharyngeal samples were collected for each episode for the detection of an extended panel of viruses. Of a total of 984 episodes, 33% were LRTIs, 29% of which were wheezy LRTIs, likely encompassing cases of bronchiolitis. Viruses were isolated in 69% episodes, 10% of which with dual co-infections. Attributable risk of wheezy LRTI was 32% for RV and 10% for RSV, followed by PIV (5%) and hMPV (4%). Overall, RSV and RV seem to be the two most common viruses associated with bronchiolitis and LRTI in early childhood. RSV is detected more frequently from children in the hospital or ED, and RV is detected more frequently from children in the outpatient clinic setting.

Transmission and seasonality

Bronchiolitis cases follow recognized seasonal and temporal patterns of infection across settings. Most seasonal variation is attributed to the dynamics of viral transmission, mostly RSV. Respiratory viruses display various transmission patterns among humans (direct/indirect contact, droplet spray, aerosol), and their transmissibility is influenced by the environment in which pathogen and host meet.[125] Transmission of RSV is usually by direct or close contact with RSV-contaminated secretions. The virus can survive for several hours on surfaces, and for approximately half an hour on hands, with common transmission among household and child care contacts.[125] Respiratory viruses that cause bronchiolitis share a relatively short incubation period with median one to six days.[126]

In geographic regions with temperate climates, epidemics of bronchiolitis and RSV peak during winter in both the Northern and Southern Hemispheres. While epidemiological data is scarce, in Portugal bronchiolitis and RSV outbreaks usually

begin in October, peak in January and end by March or April.[127-129] In contrast, RSV activity is continuous throughout the year in warm equatorial areas, although peaks may occur during rainy seasons.[130] Epidemics frequently start in coastal areas or areas surrounded by water and then move to inland areas in the subsequent months, e.g. the US southern states typically demonstrate an earlier season onset and longer duration than other North American regions.[130,131] Knowledge of RSV seasonality can be used by clinicians and public health officials to determine when to consider RSV as a cause of bronchiolitis and when to provide RSV immune prophylaxis to high risk children. However, the extent of variation in the onset timing of RSV activity can vary between communities, even those in close proximity, during the same year or an year-to-year basis.[131] While national RSV surveillance systems have been set up in some countries or geographical regions, tailoring the timing of immune prophylaxis precisely remains difficult.[131,132]

The distribution and seasonality of bronchiolitis caused by other viruses has been described in recent years. RVs are distributed worldwide with no predictable pattern of infection based on serotype. In temperate climates, the incidence of RV-bronchiolitis and other RV-related LRTI infections peaks mostly in fall, with another peak in spring, although RV infections occur year-round.[93,123,133] These seasonal trends mimic the RV-triggered increase in asthma exacerbations each fall (often referred to as the "September asthma epidemic").[134] Peak RV incidence in the tropics occurs during the rainy season from June to October.[114] Other viruses such as PIV and adenovirus circulate nearly year-round with seasonal peaks of illness.[94,135]

Our knowledge of how viral epidemics are initiated and sustained is incomplete. Data from RSV suggests there is a complex interplay of climate factors such as latitude, temperature, humidity and UVB radiance, which may affect RSV stability in aerosols or alter host resistance.[136] Demographic and sociological factors, such as overcrowding and population density, urban or rural location, also play a role in the intensity and duration of seasons.[130] Mathematical models have been to developed to reflect, evaluate and possibly predict the transmission dynamics of RSV in seasonal epidemics.[137,138]

Pathogenesis

Despite the global impact of bronchiolitis disease, our understanding of the immunopathogenesis of this condition remains incomplete. The manifestations are likely caused by a combination of viral cytotoxicity and the host immune response to infection. The extent to which any individual factor or its correlates (e.g. viral load, genetic predisposition, or dysregulated immune response) contribute to the severity of disease has been disputed, but current evidence suggests there is a complex interplay between agent and host factors (Figure 1.2). Most mechanistic studies on bronchiolitis have focused on RSV infection. While common biological pathways have been shown to be activated across multiple respiratory viruses, some aspects of agent virulence as well as the pattern and robustness of immune response to different viruses are distinctive (e.g. influenza, RSV and RV), which may affect the acute and long-term outcomes of bronchiolitis caused by different agents.[32,139-143]

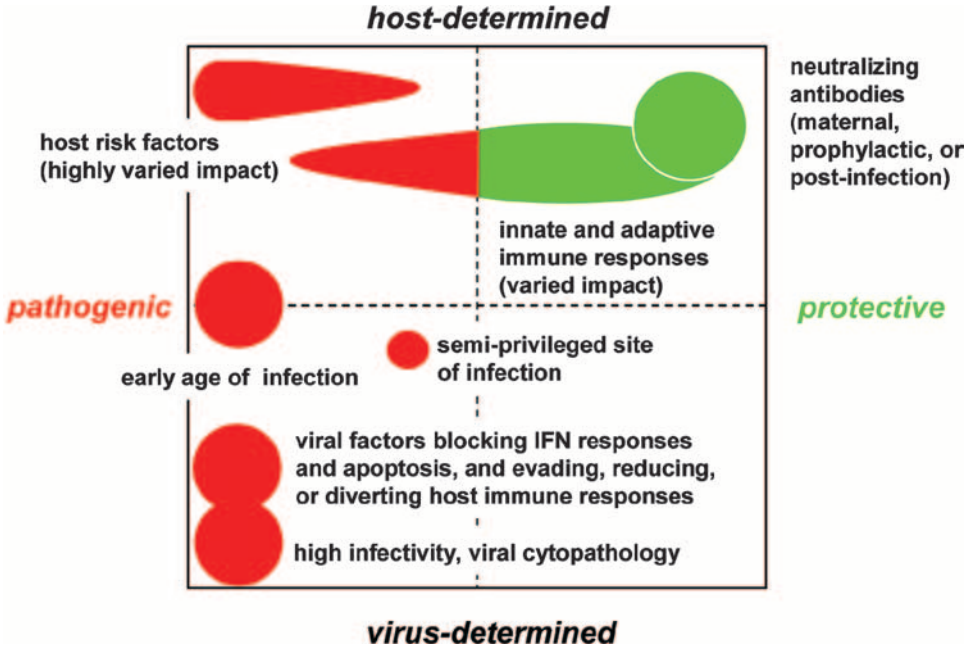


Figure 1.2. Viral and host factors in bronchiolitis pathogenesis (based on RSV model) (from: Collins, with permission)

It should be noted that translational research studies in bronchiolitis are limited by ethical and practical reasons, particularly given the need for airway biological specimen collection. Many of the studies were performed in children that were

intubated and mechanically ventilated, from whom respiratory samples of the lower airways could be more easily obtained, and the immunopathogenesis of milder disease is largely inferred.[144] Autopsy and biopsy findings are also scarce, although they provide important insight into the mechanisms of severe disease.[141,145,146] Further, findings from human studies are confounded by factors such as co-morbidity, bacterial and viral co-infections, and concomitant treatments. Animal models of viral disease complete our understanding of the pathophysiology of human disease, and are also a critical step in preclinical testing of the effectiveness and safety of new pharmacological approaches and vaccine strategies.[147] Current animal models of RSV infection include chimpanzees, sheep, cotton rats, and mice.[147] However, there are limits in the extrapolation of evidence from animal models.

Respiratory viruses causing bronchiolitis have a direct cytopathic effect on respiratory epithelial cells. In the case of RSV, the virus replicates in nasopharyngeal epithelium and then spreads to the lower respiratory tract one to three days later, by both direct spread and aspiration of nasopharyngeal secretions.[148,149] Evidence of RSV deposition in distant organs has also been shown in the myocardium, liver, and cerebrospinal fluid.[150] Ciliated cells of the small bronchioles and type 1 pneumocytes in the alveoli are major targets of infection in the lower airway, although dendritic cells, neutrophils and other cells are also infected.[145,146,151-154] The RSV virion consists of a nucleocapsid packaged in a lipid envelope derived from the host cell plasma membrane. The RSV envelope contains three viral transmembrane surface glycoproteins: the large attachment glycoprotein G, the fusion protein F, and the small hydrophobic SH protein.[154] RSV has a tropism for superficial cells of respiratory epithelium, which it infects by attaching to the cell surface through protein G, while the F protein mediates fusion with the epithelial cell membrane along with adjacent cells.[155] This (rarely) results in the formation of giant multi-nucleated cells – syncytia – for which the virus is named.[154] The SH protein may play a role in both syncytial formation and blocking of cell apoptosis, or inflammasome activation.[155,156] Virion assembly occurs at the plasma membrane of infected cells, and virions are released by budding. RV is also known to directly infect lower respiratory airways, as are other bronchiolitis associated respiratory viruses.[157-159]

It is known that influenza relies on rapid evolution and RVs on extensive diversity to escape immunity, thus influencing pathogenicity, and there is also evidence that RSV strain may contribute to differences in viral pathogenicity.[143,157,160] However, even antigenically similar or identical RSVs can be be equally strongly pathogenic. Importantly, it has been suggested that RSV is more virulent than other respiratory viruses, as multiple studies have shown that higher RSV loads are predictors of increased disease severity.[161-166]

The observations on the critical importance of the agent's virulence challenge the long-standing immunopathology-based pathogenesis paradigm of an over-exuberant immune cascade of inflammatory mediators and cellular infiltrates in bronchiolitis solely due to an exaggerated immune response.[162,167] Recent findings suggest that the response to most respiratory viruses involves a complex interplay between dysregulated innate and adaptive immunity, as well as mucosal inflammation.[142,168,169] The innate immune response has a critical role in the initial stages of infection, and innate immune mediators influence both early inflammatory responses and the subsequent development of an adaptive immune response.[168,170,171] Viral double-stranded respiratory virus RNA is recognized in epithelial cells and resident mucosal immune cells (e.g. dendritic cells and macrophages) through the recognition of pathogen-associated molecular patterns that activate various pattern recognition receptors, such as toll-like receptors and RNA-helicases, and the inflammasome.[171] A range of pro-inflammatory cytokines, chemokines and growth factors are released that promote inflammation, direct recruitment and activation of immune cells, and initiate anti-viral responses including type I/III interferons.[168,171-173] Dendritic cells and airway epithelial cells can link with adaptive immunity by activating antiviral effector and memory T-cells, or directly B-cells.[174] Neutrophils account for the majority of cells recruited into the airways of children with bronchiolitis, with lymphocytes representing up to 10%, mostly B cells.[141,175]

While the innate immune system was originally thought to be at full strength at birth, recent evidence demonstrates its immaturity in the healthy neonate. Studies addressing the role of innate immunity on RSV disease severity have had somewhat inconsistent results, possibly due to differences in study design (e.g. settings, severity, outcomes) and measurements (e.g. stimulated vs unstimulated mediators,

location of specimen collection).[172,176-187] However, evidence is accumulating that severe RSV bronchiolitis is associated with hypo-responsive innate immune function.[32,140,146,153,176,178,179,187-189] For example, non-structural proteins of RSV, expressed in great abundance in the earliest phase of infection, are capable of inhibiting host type I interferon responses.[190] The profile of innate immune dysfunction may differ quantitatively and qualitatively between viruses.[32,188] For example, recent studies investigating RSV and hMPV suggest that they elicit unique cytokine profiles, and use different mechanisms to activate human dendritic cells.[183,191-193] This could contribute to differences in bronchiolitis pathogenesis depending on agent. Growing evidence has also demonstrated an important participation of several components of the pulmonary surfactant, particularly proteins SP-A and SP-D, in the mechanisms of lung innate immunity against RSV infection, and in the onset of the inflammatory response that follows infection.[194] Infants with RSV bronchiolitis are deficient in surfactant, both in content and function.[194-197]

The dual role of the adaptive immune response in RSV bronchiolitis has also been subject of ongoing controversy, with debate on whether it protects against disease or instead causes symptoms by a vigorous inappropriate response.[142] Cellular immunity plays a role in combating and recovering from RSV infection, with CD8 T-lymphocytic stimulation and response implicated in viral clearance.[169] Humoral immunity can also have a protective role, since RSV-infected infants with higher levels of maternal transplacentally transferred anti-RSV antibodies have been shown to have a lower risk of hospitalization and reduced severity of bronchiolitis.[56,198,199] Further, passive immunization with the humanized anti-RSV F protein monoclonal palivizumab is effective in reducing disease severity in high-risk infants.[200] Neutralizing serum antibodies are an important mediator of protection against lower respiratory tract disease, whereas local mucosal IgA responses may be important in mediating protection against infection in the upper respiratory tract.[201] However, it is now clear that an immature, weak adaptive immune response during infancy is a key factor in the pathogenesis of severe RSV infection, with a possible effect of RSV on T cell depletion and suppressed cell-mediated immune responses.[141,146,176,179,202,203] Studies in patients with severe RSV bronchiolitis show a profound decrease in local interferon II production, which is associated with disease severity. Further, RSV infection elicits an antibody response that fails to establish long-lasting

immunity and prevent periodic, albeit less severe, reinfections throughout life.[56,204,205] Antibody responses are of low magnitude and poor durability, most likely due to a combination of immunological immaturity and the suppressive effect of maternally transmitted transplacental antibody.[199,206,207] Concurrently, there is evidence that T cells enhance disease. A balance between specific T cell subset function, such as Th17 cells and Tregs, may be involved in the regulation of protective immunity, immunopathology and mucosal inflammation during RSV infection.[169] Despite extensive literature on the relative balance and possible skew between Th1 and Th2 cytokine signatures, it remains unclear what if any effect this has on RSV-induced disease severity.[203]

The complex links between innate and adaptive immunity are revealed through the understanding of enhanced respiratory disease that affected children immunized with a formalin-inactivated vaccine against RSV in the 1960s. The vaccine was immunogenic, but it elicited a non-protective antibody response.[29] The main clinical manifestations were bronchoconstriction and severe pneumonia, with non-protective antibody complexed with virus deposited in affected tissue.[208] Only recently studies were able to show that immunization with formalin-inactivated RSV elicited a low-avidity, RSV-specific antibody response, a robust RSV-specific CD4+ T cell response and no cytotoxic CD8+ T cell response, due to failure to engage and activate innate pattern recognition receptors.[209,210]

In summary, the current picture of bronchiolitis pathogenesis involves a complex dysregulated immune response to viral infection. Immature and impaired innate and adaptive immune responses may interfere with the development of effective antiviral clearance, while misdirected cytokine responses and excess inflammation potentiate disease. Thus, severe RSV disease sequelae may be associated with an inappropriate immune environment, and heterogeneity in agent-specific virulence and host responses are only starting to be uncovered.

The complexity of agent-host interactions is magnified by the polygenic nature of host genetic factors related to RSV severity. Studies using the candidate gene approach have identified specific loci that likely affect RSV disease severity, and genetic associations with single nucleotide polymorphisms in genes within plausible biological pathways have been reported (e.g. innate host defense genes,

cytokine or chemokine response genes, and altered Th1/Th2 immune responses).[211-213] This has allowed, for instance, to focus on relatively novel factors of disease pathogenesis for which epidemiological data is also emerging, such as the interplay between vitamin D, its receptors and downstream innate biomarkers like cathelicidin.[211,214-217] However, methodological limitations of candidate gene approaches are well known, and larger-scale genome-wide association studies are yet to be conducted and replicated.[218] Bronchiolitis research has lagged behind in using the emerging fields of "-omics" to improve our understanding of disease pathogenesis, in contrast to their exponential use in recurrent wheezing and asthma research.[219-221] Only recently have the first high-throughput screening studies of host transcription profiles and gene suppression in animal models and humans begun to provide insights into the host response to, and regulation of, RSV infection.[32,222,223] Other genomics, metabolomics and proteomics approaches may soon follow.[224] A systems biology approach, which involves integrating information from all levels of structure and function of the system, will likely provide greater insight into the understanding of acute bronchiolitis and its links to recurrent wheezing and asthma.[225] Further, biosignatures and integrated pathways may open new avenues for the discovery of biomarkers to identify phenotypes, to confirm diagnosis and establish prognosis, and to identify therapeutic and preventative targets. However, these expectations will have to match the many limits of validity of "-omics" research.[226,227]

Pathophysiological mechanisms

The pathological mechanisms of bronchiolitis lead to loss of cilial motility and impaired mucociliary clearance, submucosal edema with bronchiolar and peribronchiolar inflammation, increased mucus secretion, neutrophilic infiltration, necrosis and sloughing of respiratory epithelial cells of the small airways.[6,141,145] The main physiological phenomena that ensues is small airways obstruction; critical narrowing of peripheral airways results in severe obstruction, with markedly increased respiratory system resistance (Figure 1.3).[228-230] Whether there is associated bronchospasm is a matter of debate. The impact of airways obstruction is amplified by developmental changes in airway diameter and compliance, pulmonary parenchyma and chest wall properties in infants, which interact in a highly complex and dynamic manner.[231-236]

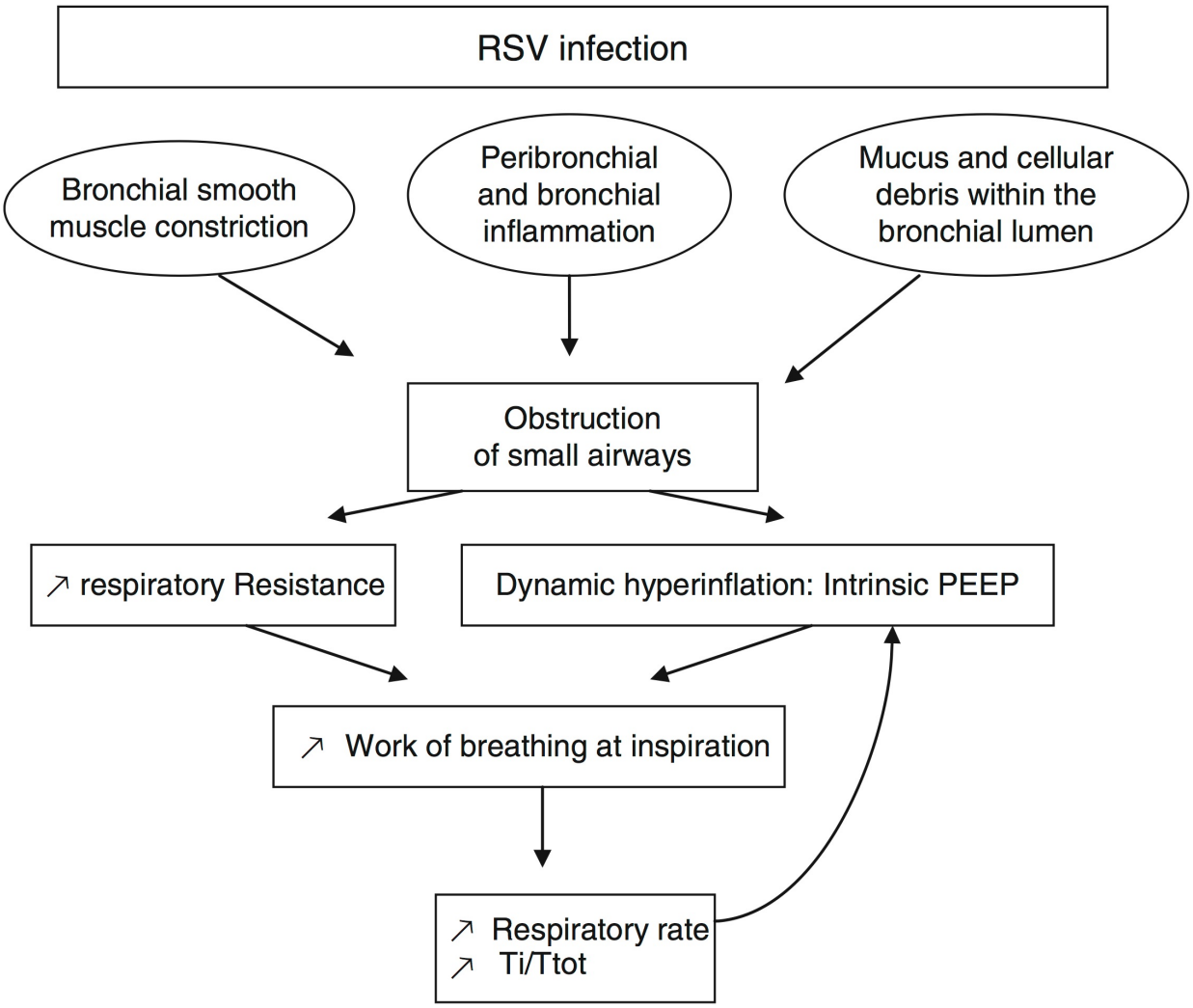


Figure 1.3. Pathophysiology of the classic form of bronchiolitis/RSV infection (adapted from: Javouhey, with permissions)

Obstruction is caused both by a ball-valve mechanism due to intraluminal plugging by mucus and cellular debris, and by dynamic small airways narrowing with disproportionate turbulence and decreased airflow during expiration.[144] The small caliber and high wall compliance of the developing airways render the infant and child more vulnerable to the development of airway obstruction. Under normal breathing conditions the small caliber of the airways represents no mechanical disadvantage because there is good correspondence between airway cross-sectional area and gas flow. When obstruction develops, however, airway resistance increases as an exponential function of the reduction in airway diameter, according

to Poiseuille's law.[237] In young infants, peripheral airways may contribute as much as 50% of intrathoracic resistance.[238] Further, airway compliance is likely greater in previously healthy infants than in adults, due to changes in the mechanical properties of the airway walls with increased collapsibility.[239,240]

Small airways obstruction leads to air trapping and areas of atelectasis.[144] Air trapping causes patchy hyperinflation, with breathing at a higher lung volume because of a raised functional residual capacity (FRC) despite reduced total lung capacity.[228-230] Hyperinflation generates an extra elastic load from the chest wall and forces respiratory muscles to operate in unfavorable biomechanical conditions, with stiffer lungs and decreased lung compliance worsened by atelectasis.[241] Risk of atelectasis is also increased because the pores of Kohn and other inter-alveolar pathways are not well developed in infants, so collateral ventilation is less effective.[235] Moreover, changes in surfactant lipid components and hydrophobic proteins impair the reduction of the surface tension at the alveoli and terminal bronchioles, resulting in decreased surface activity, atelectasis, and decreased lung compliance.[194] Infants must increase their work of breathing by using accessory respiratory muscles, which has been confirmed by various studies using different techniques to measure the respiratory muscle load in severe bronchiolitis.[229,242,243] Nasal obstruction, which is commonly present in the context of bronchiolitis, adds to the work of breathing, since a large percentage of airway resistance in infants is nasal in origin.[233] Further, infant head and neck anatomy is prone to upper airway obstruction.[244] When total work of breathing against compliance and resistance is summated, an optimal respiratory frequency exists that minimizes the total work of breathing. In obstructive lung diseases with increased resistance such as bronchiolitis or asthma, despite tachypnea, the optimal frequency is theoretically relatively decreased leading to slower, deep breathing, as compared to the rapid, shallow breathing of restrictive lung diseases such as pulmonary edema, pneumonia, or acute respiratory distress syndrome (ARDS).[245] However, in response to the higher inspiratory load, there is an increase in the inspiratory time/total respiratory time ratio and a shorter expiratory period due to airway obstruction.[246] This compounds dynamic hyperinflation and intrinsic positive end-expiratory pressure (PEEPi), which further decreases compliance and increases inspiratory work of breathing.[242,243,246]

All the aforementioned factors contribute to mismatching of ventilation and pulmonary perfusion giving rise to arterial hypoxemia. Other developmental changes in airway-parenchyma and lung-chest wall interactions also adversely affect the mechanics of breathing. Alveolar multiplication is largely a postnatal event, and infants have fewer alveoli and fewer alveolar attachments to airway walls than older children.[235] A compliant chest wall offers little outward recoil to the respiratory system and thus the elastic characteristics of the respiratory system approximate those of the lung, which has an inward recoil only slightly less than that of adults.[233,247-249] Since FRC is determined by the balance of lung and chest wall recoil forces, a high chest wall to lung compliance ratio leads to a low mechanically determined FRC relative to older children and adults.[233] Bronchiolitis is a condition with elevated closing capacity (i.e. the volume of gas that remains in the lung when small alveoli and airways in dependent regions of the lung are collapsed or considered closed), which can easily rise above FRC, therefore some lung units are closed during breathing; these areas of collapse add to ventilation and perfusion inequality and intrapulmonary shunting.[245] This is partially compensated by dynamic mechanisms that elevate the end-expiratory volume, such as modification of breathing patterns and rates.[233,250] However, these dynamic compensatory mechanisms are highly depend on sleep state, which can adversely affect the mechanics of breathing in children, e.g. with worsening hypoxemia during sleep in bronchiolitis patients.[245,251] All these deleterious developmental changes are intensified in preterm and younger infants, which are known prognostic factors for disease severity in bronchiolitis.[234,252-254]

Respiratory failure and hypercapnia in bronchiolitis is usually the result of worsening lung compliance, airway resistance and respiratory muscle fatigue. Developmental factors also contribute to the latter. Chest wall muscle contractions are needed to stabilize the compliant infant rib cage, and increased diaphragmatic work of breathing leads to significant expenditure of calories and risk of fatigue.[233] Respiratory muscle fibbers are still developing, and the proportion of fatigue-resistant fibbers is relatively low.[255] Further, a significant portion energy is wasted through the distortion of the highly compliant rib cage during negative pressure generation from diaphragmatic contraction.[235,256]

Apnea is commonly found in bronchiolitis and RSV infection, but its mechanisms are not completely understood.[257] Immaturity of central ventilatory centers is likely to be one of the explanations, which can explain the high prevalence of apnea in infants born prematurely and infants below two months of age.[258] Younger and premature infants have been shown to be vulnerable to viral and inflammatory induced central autonomic dysfunction.[259] Aforementioned changes in breathing mechanics may also contribute to apnea secondary to severe lung disease, which occurs as the consequence of continued high work of breathing and muscle fatigue.

Some evidence suggests severe bronchiolitis and RSV-associated lower respiratory tract disease encompass distinct patterns of respiratory failure. Hammer and colleagues showed that the most frequent pattern is obstructive airway disease, which could be labelled as "classical" bronchiolitis, characterized by increased airway resistance, air trapping, reduced lung capacity, and low respiratory system compliance compared with normal values, with bilateral perihilar infiltrates and hyperinflation on chest radiography.[230] A smaller proportion of children have a resistive profile, closer to pneumonitis and/or acute respiratory distress syndrome, with very low compliance and resistance, and bilateral alveolar consolidations on radiography. The latter pattern is more severe and evolves with large intrapulmonary shunt and a propensity to fluid retention or heart failure. Other authors have suggested that these patterns cannot be strictly dichotomized and form a continuum, with more severity at each end of the spectrum.[260,261]

Risk factors and prognosis

The majority of the children who develop bronchiolitis are healthy term infants without any known predisposing factors.[65] Further, as previously described, most children have a mild course, only approximately 2% to 3% will be hospitalized, and less than 1% will be admitted to an ICU, intubated, or die. Observational studies have identified demographic, environmental, family history factors and comorbidities associated with incidence of bronchiolitis of different severity, some of which restricted to RSV bronchiolitis. These factors are summarized in Table 1.1. The magnitude of the associations varies, and interactions likely occurs between different factors.

Table 1.1: Selected risk factors and prognostic factors of bronchiolitis

Factors		Risk factors for bronchiolitis incidence			Prognostic factors for bronchiolitis severity		
		Case definition			Outcome		
		All cases	*Inpatient*	*Predictive models for RSV hospitalization**	*Admission during ED visit*	*Length of hospital stay*	*Respiratory support/ mortality*
Demographic	Male sex	Yes	Yes	Included in some models	No	No	Variable
	Younger age	Yes	Yes	Included (age at the beginning of RSV season)	Yes	Yes	Yes
	Crowding/ siblings	Yes	Yes	Included	No	No	No
	Daycare	Variable	Variable	Included in some models	Variable (inverse)	No	No
	Ethnicity (white)	Variable	Variable	Not included	Variable	Variable	Variable
Personal or family history	Atopy/ wheezing/ asthma/ eczema	Variable	Variable	Included in some models	Variable	No	No
	Low SE status and maternal age/ education	Variable	Yes	Not included	No	No	No
Environmental	Tobacco smoke exposure (pre/ postnatal)	Variable	Yes	Included in some models	Variable	Variable	Yes
	Breastfeeding	Yes	Yes	Included in some models	No	Variable	No
Comorbidities	Prematurity / birthweight	Yes	Yes	Included in some models	Variable	Yes	Yes
	BPD/CLD/ history of intubation	Unknown	Yes	Not included	Yes	Variable	Variable
	CHD	Unknown	Yes	Not included	Likely yes	Variable	Yes
	Down syndrome	Unknown	Yes	Not included	Likely yes	Yes	Yes

*Based on references 272,273,275-277,282,283,288

There has been considerable research in risk of disease and prognosis of bronchiolitis, albeit studies are heterogeneous in objectives and study design, and share common methodological obstacles and limitations.[262] Importantly, it is challenging to disentangle factors associated with bronchiolitis incidence in

children without the condition (i.e. "risk factors"), from factors associated with disease severity in children who already have bronchiolitis (i.e. "prognostic factors").[262,263] While it is likely that some factors overlap, clarification on their distinct contribution to the incidence and severity of disease is scarce, as studies vary in their start points (populations) as well as their endpoints (clinical outcomes). Studies often focus on selected population subgroups, either likely to be at risk (e.g. pre-terms of a certain gestational age range) or recruited in specific settings (e.g. infants attending ED), with few population-based studies or studies in mild disease. Outcome definition and ascertainment also varies, often meshing measures of incidence and severity, e.g. restricting bronchiolitis cases to those with hospitalization, or encompassing different manifestations of LRTI. Moreover, most literature is focused on RSV bronchiolitis and LRTI, with the purpose of identifying children likely to benefit from preventive use of palivizumab. However, the direction and magnitude of association of several risk and prognostic factors may differ between viral agents. Finally, prognostic studies in bronchiolitis suffer from limitations in the design, analysis and reporting which are common in prognosis research, such as limited replication and small sample sizes, which limits the reliability and applicability of the published findings.[264,265]

It is important to consider that risk and prognostic factors are not necessarily in the causal pathway to incidence and disease severity, as most are merely associated with the "true" causal factors.[266] Estimating causal effects from observational studies has many challenges, particularly given confounding and selection biases, as well as effect modification between different factors; recent methodological developments in causal inference methods have been scarcely applied in the fields of bronchiolitis and wheezing disorders. Given the uncertainties regarding agent and host contributions to bronchiolitis pathogenesis, it is not surprising that results from epidemiological and prognosis studies have often informed further mechanistic studies. For example, epidemiological studies identified Down syndrome as a novel epidemiological risk factor for severe disease, with subsequent translational studies trying to identify specific underlying respiratory or immune susceptibilities.[267,268] Conversely, structured approaches using biological rationale to inform possible potential prognostic factors are less frequent, albeit increasing. A recent study focused on a putative prognostic candidate biomarker, cathelicidin, based on the role of innate immunity and vitamin D pathways in bronchiolitis

pathogenesis.[216] It should be noted that the fact that one or more prognostic factors are not in the causal pathway does not lessen their relevance in predicting disease and severity.

Prognostic research includes also includes the development, validation, and impact of statistical models that predict risk of a future outcome, i.e. prognostic models.[269] Prognostic models use multiple prognostic factors in combination to estimate outcome risk for an individual, based on their specific set of prognostic factor values, and can form the basis of clinical prediction rules.[270] Only recently has there been progress in the development of prognostic models and clinical prediction rules for bronchiolitis or severe bronchiolitis, mostly focused on the prediction of RSV disease with hospitalization in larger groups of late preterm infants or healthy term infants.[57,271-277] These models often include host and environmental factors such as daycare attendance and/or presence of school-aged siblings, whose prognostic value is dependent on the geographical region and socio-cultural habits. Further, considerable heterogeneity in baseline risk of hospitalizations, which is a crucial outcome in these models, is likely due to country-dependent medical practices and hospitalization guidelines.

Age, time of birth and gender are important risk and prognostic factors in bronchiolitis and RSV LRTI. In most studies, males have an increased risk of bronchiolitis with or without hospitalization as compared to females, and may also have increased mortality.[37,47,60,81,278,279] When considering all cases regardless of setting or viral agent, population-based studies show bronchiolitis is more frequent within the first six months of life.[1,47] Age distribution of incidence rates varies according to severity: hospitalizations are more frequent within the first two to three months and then decline, while outpatient visits are frequent during the first year.[47] In a recent population-based retrospective birth cohort in the UK, the median age of bronchiolitis admission was 120 days (interquartile range [IQR] 61 to 209); age of admission was slightly higher for children born preterm compared with infants born at term.[65] Further, age is a relevant prognostic factor; in a multicenter prospective cohort study of hospitalized children with bronchiolitis, age younger than two months was a strong predictor of length of stay and need for mechanical respiratory support.[5,68] Data on RSV disease shows similar findings. A narrative review of studies published before 2000 found that up to 28% hospitalized children

with RSV were aged below six weeks, and up 70% below six months, and more recent results are comparable.[54,278,280] Further, the highest incidence of RSV bronchiolitis in the community is also observed at older ages, both in developed and developing countries. In the US, stable rates for outpatient visits are seen within the first year of life, while in a prospective cohort of healthy term children in the Netherlands with active community surveillance, the median age at the time of RSV LRTI was 6 months (IQR 4 to 8).[54,57] Data from Kenya and Indonesia also found a substantial risk of outpatient severe bronchiolitis up to 18 months of age.[204,281] The risk of hospitalization due to RSV infection is higher when the first few months of life coincide with the first half of the RSV season.[57,282,283] Various causal factors have been suggested to explain the association of age and time of birth with severe disease, including exposure to viral agents, humoral and innate immunity deficiencies, and smaller airways.[284] Male–female differences in lung function may also explain the influence of gender.[285] Motives for a relatively low incidence of mild disease in healthy term infants are a matter of debate.[278,284,286]

Day care attendance, crowding, and having young siblings, are potential risk factors for bronchiolitis, thru increased risk of close viral exposure and possibly a higher viral load.[125,278] A recent systematic review explored the association of residential crowding and the risk of laboratory-confirmed RSV LRTI.[287] The association with hospitalization was consistent across risk status, study design, and geographic location, but less so for outpatient RSV LRTI. Included studies used one or more measures of crowding, including presence, number and/or age of siblings and number of people living in household. Similar associations have been found in studies analyzing all cases of bronchiolitis.[47,279] Day care attendance has shown somewhat mixed results. While it known that respiratory illness is increased in infants and young children attending day care and group care outside the home, not all studies have found a significant association with RSV infection with hospitalization.[31,57,278,282,283,288,289] Differences can likely be attributable to distinct patterns of use of daycare (e.g. very low in a Spanish cohort, while high in Netherlands and Canada), but also confounding or effect modifier variables such as presence of school aged siblings or related socio-cultural parameters.

Studies on the influence of tobacco smoke exposure on the risk of bronchiolitis and RSV LRTI have had somewhat conflicting results.[278,290] Inconsistencies in some

results may relate to different measures used to quantify smoke exposure, as well as confounding or mediation by social deprivation.[278,291] Tobacco smoke exposure is a known risk factor for many adverse health-related outcomes, and maternal smoking has been shown to reduce lung function in children, either through impairment in airway development or by changing lung elastic properties.[292-295] Two recent systematic reviews found robust evidence across study designs, patient populations, countries and methods of analysis that both pre- and postnatal tobacco smoke exposure, increased risk of bronchiolitis and RSV LRTI, particularly with hospitalization.[290,296] Results from these reviews and another study also suggested an independent association with disease severity among children hospitalized for RSV, and possibly increased risk of mortality from bronchiolitis.[290,291] In a large retrospective cohort study, Carroll et al also found a significant dose-response relationship between the number of cigarettes smoked during pregnancy and clinically significant bronchiolitis.[279] Evidence was less consistent of an association between tobacco exposure and outpatient mild RSV LRTI.[290]

Breastfeeding has a well-documented beneficial effect against many infectious diseases, and previous systematic reviews of observational studies have documented a decrease in the risk of hospitalization due to LRTIs from both developed countries and developing countries.[297-299] Different LRTIs are often aggregated in these studies, and data is less clear as to a specific protective effect of breastfeeding against bronchiolitis and RSV infection.[278] Studies in cohorts of late preterm infants showed mixed results, but evidence seems to suggest reduced incidence and possibly severity of bronchiolitis and RSV infection.[57,282,283,288,300,301] Further, it is likely that breastfeeding interacts with other risk factors like weaning, crowding, daycare attendance, smoke exposure and socioeconomic status, and methodological limitations of most studies have been unable to address these interactions.[299,302] Evidence is conflicting regarding the association of breastfeeding and wheezing illnesses and asthma, and reviews have often encompassed bronchiolitis as an outcome, which may be an additional source of bias.[303-306] There are many biological reasons why breastfeeding would protect against bronchiolitis, including transfer of maternal antibodies, presence of antiviral and lung maturation mediators in milk, and its innate immune modulatory effects.[278,307]

Other sociodemographic factors have been associated with bronchiolitis incidence and severity, but results are somewhat conflicting. These include urban environments, low socioeconomic status, and low maternal age and education.[47,53,57,278] Certain clinical populations seem to be at higher risk of severe bronchiolitis with hospitalization, including native or aboriginal North American, American Samoan and Bedouin infants.[47,308-313] Contradictory data exists regarding the risk of hospitalization, disease severity and outpatient visits for hispanic, black and white infants.[53,81,278] Differences between ethnic groups are most likely attributable to associated socioeconomic determinants, health-seeking behaviors, or access to care, although they may also relate to genetic factors.[313]

Traditional, well-established, high-risk populations with clinical comorbidities at increased risk for severe RSV bronchiolitis and LRTI, include preterm infants with or without underlying bronchopulmonary dysplasia (BPD)/chronic lung disease (CLD) of infancy, and children with hemodynamically significant congenital heart disease (CHD). Evidence from the pre-palivizumab era consensually identified prematurity and BPD/CLD as distinct independent and interacting risk and prognostic factors for severe RSV bronchiolitis and LRTI disease, despite heterogeneous study design and quality.[31] Infants with hemodynamically significant CHD have also been shown to be at increased risk of severe RSV and all cause bronchiolitis, as well as to have increased severity of disease and mortality.[31,65,314,315] As palivizumab use for these subgroups increased differentially in multiple countries and health systems, causal and prognostic associations with RSV disease are now harder to infer. Importantly, RVs are also emerging as important agents of bronchiolitis in very low birth weight infants, particularly in children with BPD/CLD.[115] Recent studies continue to identify prematurity and/or BPD/CLD as risk factors for RSV-related but also all-cause bronchiolitis hospitalizations, with likely impact on disease severity (i.e. length of stay, need of ventilatory support and mortality).[5,31,54,65,68,289,314] Risk of bronchiolitis in both premature and BPD/CLD patients may extend longer during the first year than for term infants.[65,77] Whether stratified levels of BPD/CLD severity modify the magnitude of risk of bronchiolitis is not clear.[77,316] Most focus during the last decade has been on late preterm infants (33 to 35 weeks of gestational age) with no additional comorbidities, a subgroup particularly at high risk of RSV-hospitalization.[31,317,318] Some of the above mentioned individual and environmental risk factors have been included in prognostic models aiming to identify children in

this age range at higher risk of RSV infection with hospitalization; these models have been derived and externally validated in Europe and North America.[272,273,275-277,282,283,288] Low birth weight is also consistently independently associated with bronchiolitis visits and admissions even in term infants; it is also a prognostic factor for need for ventilatory support.[47,68,319] Lung development and immune susceptibilities likely explain the role of prematurity and BPD/CLD, while intrauterine growth restriction may also have an independent role, alongside potential confounders (e.g. maternal smoking).[233,236,254,294,320] Direct cardiac evolvement reported during RSV infections ranges from arrhythmias to global myocardial involvement with mechanical dysfunction, and anatomical cardiac lesions can worsen an already compromised respiratory status.[321-323]

Recent studies have focused on the role of other chronic conditions, both congenital and acquired, as independent risk factors for bronchiolitis.[324] A range of chronic neurological diseases, including neuromuscular diseases, cerebral palsy, and epilepsy, have been shown to increase both the risk of hospitalization and the risk of a complicated course.[65,314,324,325] In children with technology dependence and neuromuscular disorders, multiple pulmonary, immune and neurological factors contribute to increase their risk of clinical deterioration due to airway infections.[326] Primary or acquired immune deficiency syndromes and other conditions with immune suppression, including solid organ or hematopoietic cell transplantation, have been associated with prolonged viral shedding and increased morbidity and mortality rates in bronchiolitis and RSV infection.[31,324,327-330] In children with cancer, chemotherapy-induced lymphopenia is a prognostic factor for severe disease.[331] It should be noted that all respiratory viruses may lead to severe respiratory disease and respiratory failure in immunocompromised children, and the clinical phenotype may be indistinguishable between bronchiolitis and pneumonia/pneumonitis.[327] Down syndrome has emerged recently as a novel risk factor for disease incidence and severity.[65,267,317,324,332,333] Both lung, airway and immune abnormalities may explain this increased susceptibility, which is independent from Down syndrome comorbidities such as prematurity and CHD.[268,334,335] A set of other chronic conditions, including cystic fibrosis, abnormalities of the airway and other types of CLD, chromosomal and genetic disorders, and congenital malformations, have been linked to a higher risk of hospitalization for RSV LRTI and possibly disease severity.[65,324] More research is needed to clarify

whether these associations are due to specific susceptibilities, as opposed to confounding by admission threshold, misclassification of conditions, or other biases.[324] Evidence also suggests that nosocomial/hospital-acquired RSV infection is an additional major risk factor for death in children with severe RSV infection, particularly those with chronic comorbidities.[78,336,337]

While it remains unclear if the viral cause of bronchiolitis is a clinically relevant prognostic factor, recent studies seem to suggest an association with disease severity, particularly in the case of co-infections.[5,338-340] The largest multicenter study of hospitalized bronchiolitis found an association of viral agent with length of stay, but not with need for ventilatory support.[5,68] In particular, children infected with RV alone or in combination with non-RSV viruses had a significantly shorter length of stay, while for RSV/RV co-infections it was significantly longer. RSV is known to induce changes in airway epithelial cell adhesion molecules and to reduce interferon II response, both of which may enhance RV replication and explain the increased severity of illness of RSV/RV co-infections.[5] However, other studies have found contradictory results on the prognostic value of co-infections using measures of disease severity such as hospitalization rate, length of hospital stay, symptoms, severity score, or duration of illness. Further, findings of a milder course for RV-alone bronchiolitis have been replicated by some, but not all related studies.[91,123,133,341,342] Evidence is scarce about other co-infections, with some studies suggesting that combinations of RSV with hMPV or BoV may be deleterious; more research is needed to determine the clinical implications of the many other pathogen combinations.[338-340,343] As previously mentioned, RSV viral load may affect disease severity.[161,163-166] Data are conflicting regarding the influence of RSV serotypes, while RV groups are also being studied.[114,163,344-347]

The association of bronchiolitis with family or child history of allergic disorders, asthma and atopic dermatitis, is complex and likely bidirectional.[278,348,349] Results of studies of these variables as predictors of bronchiolitis incidence and severity have been conflicting, and will be further discussed in the section about bronchiolitis, recurrent wheezing and asthma, along with genetic predictors and neonatal lung function.

CLINICAL PRESENTATION, DIAGNOSIS AND NATURAL HISTORY

Clinical findings

Acute viral bronchiolitis is a clinically diagnosed condition based on a constellation of symptoms and signs.[2,3,350] Diagnosis is usually straightforward during the epidemic months, but there is considerable variability in clinical findings and in their interpretation, both over time and between patients. Recent large multicenter cohorts have improved our understanding of how clinical findings on presentation can discriminate disease severity and predict clinical course, but considerable limitations remain in discriminating disease severity and predicting clinical course.[5,262,351,352]

Bronchiolitis is often preceded by a one- to three-day history of upper respiratory tract symptoms, such as nasal congestion and/or discharge and mild cough.[2,3,350] In most instances, the patient's history reveals exposure, either to an adult or older child with a common cold or other trivial respiratory tract infection, or to an infant with similar diagnosis at home or in the daycare setting. The onset of lower respiratory tract symptoms is usually relatively quick, often being recognizable from the caretaker's description of the illness. The maximum severity of illness generally is attained within 24 to 48 hours of the first signs of lower respiratory tract illness.[353] The physical examination reflects the dynamic nature of the disease, and serial observations are required over time to fully assess the child's status. Manifestations may range from mild signs of respiratory distress to respiratory failure. There are variable degrees of tachypnoea, tachycardia, and signs of increased work of breathing, including flaring of the alae nasae, grunting, supraclavicular, subcostal, and intercostal retractions, use of accessory muscles and abdominal breathing. In severe cases cyanosis may occur. Upper airway obstruction may also contribute to work of breathing.

The predictive value of physical examination correlates of respiratory distress in bronchiolitis is not similar between different individual clinical findings nor consistent across studies, and has been rarely systematically studied.[262,354,355] There are likely limits to the validity of static measurements in a highly dynamic condition, and different measures may reflect overlapping but distinct dimensions of respiratory distress related to airway and pulmonary involvement due to disease progression and severity. Further, the presence and magnitude of treatment response

(e.g. to some bronchodilators) and post-treatment values may also have distinct prognostic significance.[356]

Some studies across outpatient and inpatient settings have found an association between absolute values of respiratory rate and/or presence of tachypnea and increased risk for severe disease, as assessed by hospitalization rates, length of stay and need for ventilatory support.[352,357-362] However, others studies from similar settings and populations did not report such findings.[5,68,356,363,364] It must be noted that there are many sources of within-patient variability when measuring respiratory rate in young children (e.g. fever, activity status, setting) and that the accuracy of measurement methods varies.[365-373] Further, respiratory rate changes over the first year of life and between patient variability is considerable in this age range.[369,374] Evidence-based normative values for respiratory rate in healthy infants have only recently been obtained, and tachypnea thresholds are heterogeneous, being a possible cause of misclassification.[372,374,375] The situation is similar for values of heart rate, which have been rarely associated with bronchiolitis disease severity.[356,360,361] Thus, the predictive value of such vital signs is probably best shown incorporating both their continuous nature and the effect of various confounders.

Clinical findings of work of breathing have also been associated with disease severity in some studies.[5,68,356,376] In a recent multicenter prospective cohort of hospitalized children, presence of retractions was associated with longer hospital stay, while only severe retractions were associated with need for ventilatory support.[5,68] Interestingly, this study identified a novel subgroup of children with bronchiolitis that have a rapid respiratory decline, with an increased risk of ventilatory support if difficulty breathing had begun the day of hospital admission.

On auscultation, the major findings include widespread adventitious sounds, with either or both wheezes and crackles.[2,3,350] Wheezes are continuous sounds that represent 'fluttering' of the airway walls due to airflow limitation.[377-379] Because the site of airway obstruction in bronchiolitis is variable, wheezes are polyphonic.[379] In the presence of extensive small airway narrowing, the resultant high pleural pressure swings can cause compression (inward collapse) of larger airways during expiration, producing generalized expiratory wheezing.[379] If airway obstruction is severe enough, wheezes may also occur during inspiration.[380] The volume of the

wheeze is not an indication of the degree of airway obstruction: on the one hand, wheezing is often audible without the use of a stethoscope; conversely, severe obstruction may limit flow to such a degree that insufficient energy is dissipated to induce airway wall fluttering.[380] Wheezes are traditionally high-pitched, but low-pitched adventitious sounds, i.e. rhonchi, may also be present due to secretions within the large airways.[350] Fine inspiratory crackles (i.e. rales, crepitations), which are discontinuous, interrupted explosive sounds, are also frequently heard on auscultation as units of alveoli pop open due to obstruction of distal airways with secretions.[4,377,379] A prolonged expiratory phase of breathing can occur. Few studies have assessed the value of presence and quality respiratory sounds in predicting bronchiolitis severity, to mixed results.[356,358] Moreover, a study on the validity and reliability of stethoscope examination in infants with acute respiratory disorders has shown that agreement between observers for the presence of crackles and wheezes was moderate and poor, respectively.[381] Other thoracic findings include hyper-resonance which may be detected on percussion, and chest may be full. Due to lung hyperinflation of the lungs secondary to air trapping, it is not uncommon to find a distended abdomen and palpable liver and spleen.[350]

Bronchiolitis has a widely reported association with apnea, with most literature focused on RSV-related apnea. A recent systematic review found that the reported incidence of apnea in hospitalized patients with RSV ranged from a high of 23.8% to a low of 1.2%.[257] This wide range likely reflects factors such as the use of different definitions and case ascertainment of apnea (e.g. variable duration of apneic period, method of apnea measurement) and different inclusion/exclusion criteria of study populations (e.g. regarding chronological and gestational ages, comorbidities such as neuromuscular disorders, and different levels of disease severity). Incidence of apnea incidence was higher in preterms and children with young chronologic age.[257] A trend toward decreasing rates of apnea in more recent studies was also found; in studies that excluded children with serious underlying illnesses, the incidence of apnea was less than 5%, below the often-quoted risk of 10% to 20%.[150,257] In a recent large study of hospitalized patients with bronchiolitis caused by any viral agent, inpatient apnea occurred in 5% of patients. Independent predictors of apnea included: corrected ages up to eight weeks, birth weight less than 2.3 kilograms, and having a room air oxygen saturation before admission below 90%.[382] There was also a U-shaped association between the respiratory rate

before admission and apnea, i.e. children with initial low or high respiratory rate had a higher risk of apnea. While apnea can occur early in the course of viral disease as its first manifestation (i.e. central apnea), in this cohort a considerable proportion of children had apnea after a few days of respiratory distress (i.e. apnea possibly related to failure of oxygenation and/or ventilation). In this study, the risk of apnea risk was similar across major viral pathogens, and non-RSV agents have also been associated with apnea.[382-384]

Feeding problems are very common in bronchiolitis. Vomiting can occur, and dehydration is an important consideration in the clinical assessment of the infant, compounded by presence of fever and high work of breathing.[350] Feeding or dehydration have been identified as predictors of hospital admission and length of hospital stay in some, but not all, studies.[5,68,356,376] Irritability can occur; however, the infant is rarely systemically toxic in the absence of complications.[3] Although reported numbers vary, a large proportion of infants have mild fever, but a temperature of ≥39.5 degrees centigrade is rare in uncomplicated bronchiolitis.[3,350] Few studies have identified fever as a predictor of disease severity.[385,386] Other findings include a mild conjunctivitis and pharyngitis of varied severity.[350]

Diagnostic tests and associated conditions

Pulse oximetry was introduced into clinical practice in the early to mid-1980s, and has been rapidly adopted as a simple, noninvasive, and reasonably accurate estimation of arterial oxygen saturation for the clinical assessment of children with respiratory disorders.[39] Oxygen saturation is a particularly relevant indicator of disease severity in conditions such as bronchiolitis where ventilation/perfusion mismatch and ensuing hypoxemia are key to disease progression. It has been proposed as the "fifth vital sign" in acute pediatric assessment, since clinical evaluation does not identify hypoxemia adequately.[387,388] The use of pulse oximetry use has led to significant changes in physicians' management of bronchiolitis, since decisions to initiate or discontinue oxygen therapy are mostly based on oxygen saturation values, and usually imply a decision to hospitalize or discharge at the ED, as well as the timing of discharge from hospitalized children. In turn, this can be a source of confounding when studying oxygen saturation as a prognostic factor of bronchiolitis severity, since hospital admission and length of stay are often used as outcomes of disease severity. Some studies have shown that lower oxygen

saturation values increase the likelihood of admission, and predict longer hospital stay, as well as need for ventilatory support.[5,68,352,357-361,364,389,390] However, there is conflicting evidence on these associations, depending on time of measurement and intercurrent treatments.[362,376,391,392] Importantly, thresholds of oxygen saturation that increase the risk of these outcomes vary widely in various studies (e.g. from <85% to <95%), and there is no consensus between management guidelines as to which thresholds are beneficial and safe to admit, treat, and discharge infants with acute bronchiolitis.[36,393,394] Further, various studies have shown oxygen supplementation is the prime determinant of length of hospitalization, since feeding difficulties resolve sooner than hypoxemia (Figure 1.4).[390,392] In particular, small differences in a range of values of oxygen saturation for which the predictive value and the benefit/harm of any intervention is not clear (e.g. 92 to 94%) are known to significantly influence the decision to admit/discharge at the ED, or to prolong hospitalization. This may be due to differences in physician's perceptions of benefit/risk, local practices and methods of oxygen saturation monitoring (i.e. continuous vs. intermittent, with children awake vs. sleeping).[390,395,396] A recent clinical trial that randomized patients to either true or altered oximetry, in which the true saturation measurements were increased by 3%, showed this minor elevation of modestly suboptimal saturations in bronchiolitis significantly decreased hospitalizations.[397] Two ongoing randomized inpatient trials are addressing the issues of oxygen saturation thresholds for hospital discharge, as well as intermittent vs continuous monitoring strategies.[398,399] We should note that normative pediatric oxygen saturation values have not yet been established, and there is variation in instruments used, probe positioning, measurement protocols, as well as well as with age, altitude, 24-hour cycle and sleep.[39] Moreover, oxygen saturation does not necessarily provide reliable information regarding the oxygenation status of tissues, and does not reflect ventilation or acid-base status. In particular, children with discordance between clinical findings of respiratory distress out of proportion to the pulse oximetry findings must be evaluated carefully because respiratory failure may occur precipitously.[350] While pulse oximetry has obviated the regular use of arterial blood gas sampling, it may still be useful in severe cases in which hypercarbia and low blood pH is a concern and respiratory support is considered.[246]

The clinical utility of additional routine diagnostic testing is not well supported by evidence.[400] There is significant variation in the use of supportive testing such as

chest radiography, blood counts, and specific testing to determine the viral agent of bronchiolitis in various settings.[401-407] Use of these tests has been usually justified for the following reasons: ruling out other differential or concurrent diagnoses and complications (e.g. bacterial pneumonia), assessing disease severity (e.g. degree of pulmonary involvement), or stratifying treatment, predicting prognosis or cohorting inpatients (e.g. depending on viral agent).

The issue of concurrent or complicated bacterial infection in bronchiolitis has been considerably studied. Acute otitis media (AOM) is very common, occurring in more than half of hospitalized patients with bronchiolitis.[408-410] Bacterial pathogens can be isolated from middle-ear aspirates in a majority of patients with AOM, Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis being the most frequent isolates. Mixed bacterial and viral AOM seem to be very frequent, as RSV is also isolated from middle-ear aspirates in about half of cases, but no clinical features discriminate well between viral and bacterial AOM.[408-410] About a quarter of patients have or eventually develop otitis media with effusion.[408] AOM does not seem to influence the clinical course of bronchiolitis.[409]

A systematic review of observational studies has shown that reported rates of serious bacterial infection were low in febrile infants younger than 90 days with bronchiolitis and/or RSV infection.[110] No cases of meningitis were seen and very few cases of bacteremia were reported in the studies reviewed; urinary tract infection was the only infection reported with significant frequency, although the rates might be confounded due to asymptomatic bacteriuria. Saijo et al found elevated white blood cell counts and neutrophil counts were more likely in children with radiologically-defined pneumonia than in children with bronchiolitis.[411] This and other studies have suggested a possible role for inflammatory makers (e.g. C reactive protein and procalcitonin) in identifying or excluding bacterial co-infection, defined by either positive culture or chest radiograph result.[411-413] However, data is contradictory on the diagnostic accuracy and clinical utility of these tests for the diagnosis of serious bacterial infection or pneumonia, or its predictive value for severe disease.[109,414-418] Further, existing evidence does not differentiate between children with typical or atypical (e.g. high fever) findings in bronchiolitis.[419-422] Thus routine use of these tests is currently not recommended by current practice guidelines.[36,393,394]

The radiographic appearance of the chest varies considerably in bronchiolitis. In many cases anteroposterior radiographs are normal, but they may reveal a spectrum of findings indicative of airway disease, most frequently hyperinflation (most prevalent), prominent bronchial markings and peribronchial thickening.[42,423] Multifocal patchy atelectasis may also occur, and on sequential imaging typically shows a shift in its distribution. A minority of infants with bronchiolitis have both airway and air space disease, while lobar consolidation is a rare occurrence. Some studies have reported predictors of chest ray abnormalities, e.g. presence of fever, hypoxemia, respiratory distress or inflammatory markers.[411,424-426] However, these studies usually include older children with acute recurrent wheezing who may show differences from "typical" bronchiolitis, and radiological findings are defined heterogeneously.[427] Importantly, whether the identification of such findings is clinically relevant ultimately depends on:

1. the reliability of chest radiography in bronchiolitis;
2. its diagnostic accuracy in identifying complicated disease such as bacterial pneumonia;
3. its predictive value for severe disease; and
4. its utility in changing clinical management.

There is considerable variability in reported reliability of chest radiography in acute LRTI in children.[428-433] Clinical interpretation of radiological findings is known to vary, and physicians may misinterpret atelectasis or airspace disease with infiltrates as possible bacterial infection. It is well known that children with suspected bronchiolitis or LRTI that undergo chest radiographs are more likely to receive antibiotics.[417,423,434] However, as seen before, bacterial co-infection is uncommon and likely restricted to severe disease. Predictors of physicians performing a chest radiograph (e.g. young age, retractions, crackles) are somewhat distinct from those that are associated with abnormal findings, which suggests that a combination of factors prompts the diagnosis of pneumonia.[426,434] Further, evidence is conflicting regarding an association of radiological findings with disease severity.[42,400] It is thus not surprising that most studies do not suggest a clinically relevant change in management or prognosis of children undergoing chest radiographs.[400,417,423,435,436] Chest radiograph findings may have a role in severe disease, given the aforementioned continuum between bronchiolitis and pneumonia. Current guidelines do not support its routine use, and usually restrict it for inpatients who

do not improve, if the severity of disease requires further evaluation, or if atypical findings suggest another diagnosis.[36,393,394]

The possible implications of specific viral agents in the epidemiology, pathogenesis, and short-term prognosis of bronchiolitis have been presented previously, while their importance in long-term complications will be discussed in the next section. We have also mentioned how different specimen collection techniques and viral identification methods are available. These may influence patient management depending on their diagnostic accuracy, prognostic value and speed with which results can be provided to the clinician.[122] Suggested motives for viral testing have included: reducing unnecessary treatment with antibiotics; facilitating appropriate patient placement and cohorting of patients and staff; identifying viral agents for which effective antiviral therapy is available; collecting and reporting of health care–associated (nosocomial) infection rates; identifying emerging agents; defining and tracking of epidemiological trends; and assessing the effectiveness of preventive measures, including administration of palivizumab.[437] However, the knowledge gained from point-of-care testing rarely alters therapeutic decisions or outcomes for the vast majority of children with clinically diagnosed bronchiolitis.[5] No virus-directed treatments are yet available except for influenza, and none of the currently used drugs have shown differential effects based on specific viral agent. There is still considerable uncertainty regarding the role of viral identification for the purpose of short- or long-term prognosis, thus its routine for the individual patient outside research and epidemiological monitoring studies needs to be clarified.

The issue of virologic testing for cohorting of inpatients is more controversial. Many have recommended its use with the aim of reducing the risk of nosocomial cross-infection of other patients.[71,438-440] However, there are limitations to this approach, and current guidance is conflicting as to its routine use.[36,393,394,441,442] Most hospital cohorting practices are based on point-of-care RSV testing. This is questioned by emerging evidence on the prevalence and possible prognostic value of co-infection detected by molecular methods.[5] Further, evidence is conflicting as to a benefit of physical isolation and assignment of personnel to care only for these patients when compared to other strict contact isolation measures considering the risks and modes of transmission of the agents that are most likely to infect infants during the bronchiolitis season.[71,72,314,443,444] Factors to consider may include resource

constraints, setting and patients at risk with underlying comorbidities. As new evidence accumulates, there could be marked changes in current understanding and consensus on the clinical utility of viral testing.

Extra-pulmonary manifestations may occur in children with severe RSV infection, and possibly in cases of bronchiolitis by other viral agents. Life-threatening manifestations apart from central apneas include status epilepticus, ventricular tachycardias and fibrillation, heart block and pericardial tamponade.[321,323,445] Hyponatremia is also relatively commonly reported, and in severe cases can cause seizures.[446] This may relate to the presence of inappropriate antidiuretic hormone secretion in the context of severe pulmonary disease, to the use of inappropriately hypotonic fluid therapy in hospitalized patients, or to direct central nervous system involvement by RSV.[445,447,448] Renal function and electrolytes may be measured if this is suspected, or if the infant is clinically dehydrated.

Course of disease

For children with bronchiolitis admitted to hospital, the duration of hospitalization is influenced by aforementioned prognostic factors and local practices. In particular, oxygen supplementation (primarily) and feeding practices are key mediators in determining length of stay. Once infants are hospitalized and stabilized, subsequent deterioration to the point of requiring intensive care is less frequent but may occur. Most patients can be discharged from the hospital within two to three days after admission, although length of stay varies considerably in reported studies.[10] A majority of children with bronchiolitis attending an ED are discharged home. Mansbach et al developed a comprehensive low-risk model for children with bronchiolitis, in which factors associated with discharge included: age equal or above two months, no history of intubation, a history of eczema, respiratory rates below age-specific calculated thresholds, no or mild retractions, initial oxygen saturation equal or above 94%, fewer salbutamol or adrenaline treatments in the first hour, and adequate oral intake.[449]

Despite traditional clinical teaching usually describing bronchiolitis as a short-term, acute condition, evidence suggests that a small but important proportion of infants have a somewhat protracted clinical course.[49,450-452] A follow-up observational study of hospitalized children found two thirds experienced difficulties with normal

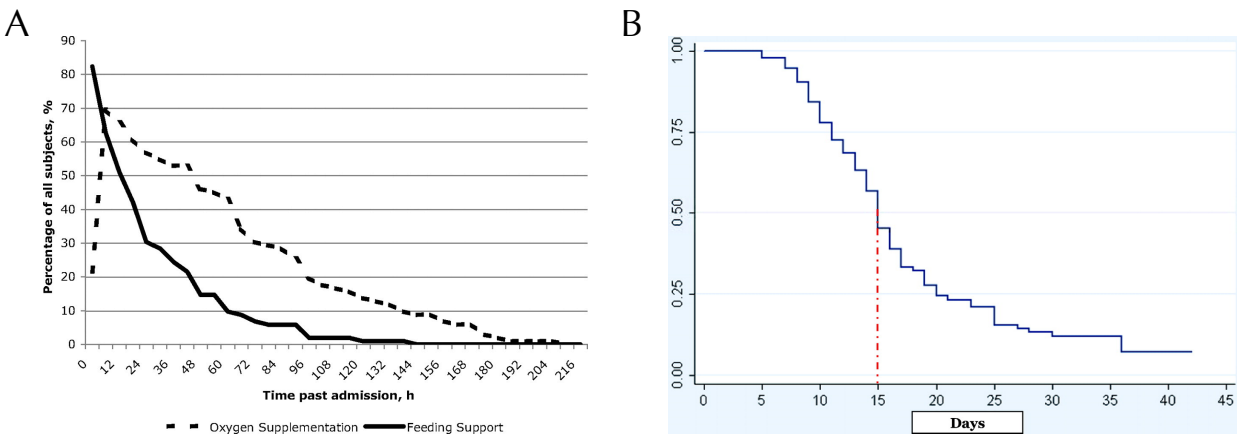


Figure 1.4. Course of disease in bronchiolitis: feeding support and oxygen supplementation for infants admitted to the acute medical wards (A), and duration of illness (based on cough) in infants evaluated in the emergency department (B; median duration of illness of shown in red) (adapted from: Unger, Petruzella, with permissions)

routines (i.e. feeding, sleeping, contentedness, liveliness) on the day of discharge.[451] A substantial proportion of infants had respiratory symptoms by two weeks, including 31% of cough, 24% trouble breathing and 22% wheezing, with feeding problems being less common. Up to one fourth of children had one or more functioning limitations five or more days following discharge, and family routines remained disrupted accordingly. Delayed recovery was associated with parental work time loss and less favorable parental impressions of care in the hospital. Infants discharged from the ED with likely less severe disease also have a prolonged disease course. Using cough as a main outcome, Petruzella et al found a median duration of illness of 15 days in infants seen in the ED, and almost 25% of the infants continued to be symptomatic three weeks after the onset of illness (Figure 1.4).[452] In this cohort, children with a history of eczema trended toward a longer median duration of symptoms, while RSV status or secondhand smoke exposure did not affect disease duration. Approximately three-fourths of working caregivers reported missed days of work or day care. Such protracted recovery often leads to unscheduled medical visits to an ED or clinic in these infants. Norwood et al found one of six children had unscheduled visits within two weeks after ED discharge, and predictors included age under two months, male sex, and previous hospitalization.[453] This proportion can reach close to 40% when considering a longer one month follow-up and all types of unscheduled medical visits.[450,452] This

recovery period is in line with biological evidence showing the respiratory epithelial cells usually recover within 2–4 days, but histologically the ciliated epithelial cells take 2 weeks to regenerate.[144] The next section will discuss later post-bronchiolitic wheezing.

BEYOND BRONCHIOLITIS: RECURRENT WHEEZE, ASTHMA, AND LONG-TERM RESPIRATORY MORBIDITY

The association between bronchiolitis, asthma and long-term respiratory morbidity has been the subject of enduring debates for decades.[348,454-459] A first episode of bronchiolitis and wheezing may be a manifestation of recurrent wheezing phenotypes with heterogeneous biological, genetic, viral or environmental determinants, and distinct prognosis.[50,460-465] A growing body of epidemiological evidence supports an association between bronchiolitis and increased incidence of subsequent recurrent wheeze and later asthma. The focus of controversy is whether viral bronchiolitis is a cause of wheeze, by affecting lung and airway structure and function or by inducing a long-term aberrant immune response to viruses or allergens (also called the serial hypothesis); or rather a first indication of long-term airway morbidity due to pre-existent host susceptibility factors such as alteration in airway function or structure or a susceptibility to develop immune responses that predispose to airway obstruction (parallel hypothesis).[142,349,458] It is likely that both mechanisms interact and contribute to asthma inception, with complex and bidirectional causal links in which the nature of the response to the different viruses associated with asthma will depend on the genetic background of the individual, on concomitant environmental exposures, and on the timing of the infectious episode, in relation to the degree of maturation of both the immune system and the airways (Figure 1.5).

Several systematic reviews of observational studies have focused on the association between RSV bronchiolitis and recurrent wheeze and asthma.[48,466] In 2000, Kneyber et al pooled data from six studies with control groups and found up to 45% of children reported recurrent wheezing until five years (defined as at least three episodes of wheezing verified by a physician, or the use of bronchodilators in the year preceding the follow-up study), as compared to only 6% in the control

group (odds ratio-OR 5.5, 95% confidence interval-CI 2.4 to 12.6).[466] However, after this period, the difference was not significant (OR 2.4, 95% CI 0.7 to 8.4). A later review by Perez-Yarza and colleagues included a considerable number of original studies and follow-up reports published in the 2000s.[48] The authors performed a qualitative assessment of methods and results but no meta-analysis. They concluded that there was an association between RSV infection and the emergence of different asthma phenotypes, with a gradient effect and progressive disappearance of this effect with increasing age. Two more recent reviews with meta-analysis provided quantitative results on the association between cases exclusively with RSV hospitalization, wheezing and asthma.[467,468] In one review, the pooled overall OR was 3.84 (95% CI 3.23 to 4.58) with moderate heterogeneity between studies (I^2 = 45%); using meta-regression, the association was found to decrease with age at follow-up, consistent with the findings of longitudinal studies.

Methodological differences between included studies likely impact the magnitude, precision and risk of bias of these findings from observational studies. All reviews have highlighted methodological limitations and poor study quality that may compromise the validity of the results. Most cohort studies are not population-based, and usually consist of a selection of "exposed" children with bronchiolitis compared to with an external comparison cohort of "unexposed" children (the label prospective case-control design is often used incorrectly). Challenges include how the cohort population and "exposure" are defined, i.e. what is the case definition of bronchiolitis (community surveillance, outpatient visits, or hospitalized cases), and how the control group is selected (i.e. what is the source population, is there matching or not). Further, outcome definition of recurrent wheezing and asthma varies (e.g. physician-diagnosed episodes, parent-reported symptoms), phenotypes may differ, as does timing of measurement, and other domains may be measured (e.g. lung function, inflammatory markers, allergic sensitization). The former factors may lead to confounding and selection biases, while the latter reflect how wheezing and asthma are heterogeneous and may encompass different phenotypes of disease.

Large studies based on twin pairs mostly support the hypothesis that the association between RSV and asthma is essentially due to genetic background, and RSV disease is an indicator of the genetic predisposition to asthma.[469-472] Arguments in favor of

pre-existent mechanisms of bronchiolitis and long-term wheeze include the presence of predisposing genetic, pulmonary, and immune susceptibilities.[142] First, we have mentioned how candidate gene studies have found diverse genes associated with increased risk of RSV bronchiolitis, including those related to airway mucosal responses, innate and adaptive immune responses, chemotaxis and allergic responses. Importantly, genetic association studies including hospitalized children have found that genes associated with early and late wheezing (i.e. at three and six years, respectively) after RSV LRTI are distinct.[473,474] This suggests that RSV LRTI hospitalization and subsequent early postbronchiolitis wheezing have different pathophysiological mechanisms from late wheezing or allergic asthma in later life.

Second, pulmonary function abnormalities underlie the risk of bronchiolitis, recurrent wheeze and possibly asthma. There is now well established evidence that indices of airway size (e.g. obtained from either partial maximal flow–volume curves or other measures of airway resistance), are inversely associated with the risk of having wheezing LRTI including bronchiolitis during the first years of life.[285] This may mediate the effect of smoking exposure in utero.[294] Further, the degree of premorbid lung function changes is associated with the severity of bronchiolitis and LRTI. Prematurely born infants hospitalized with RSV or other viral LRTIs had higher resistance of the respiratory system at birth than those who were not admitted.[475] Similarly, a recent prospective cohort of unselected term infants found differences in neonatal respiratory system compliance and resistance between infants hospitalized RSV patients compared with non-hospitalized RSV-positive infants.[476] Further, these measures of neonatal lung function were also associated with post-RSV wheeze, which was only marginally explained by any preexisting wheeze or the severity of the episode itself. Neonatal total lung resistance has also been shown to be associated with RV-induced wheeze.[477] Congenital alterations in the regulation of airway tone may also predate bronchiolitis and wheezing. Data available on bronchial responsiveness are much scantier and less conclusive than that for earlier postnatal airway function tests without provocation. A recent large birth cohort of at-risk neonates born of mothers with a history of asthma found neonatal bronchial hyper-responsiveness to metacholine, but not airflow limitation, preceded acute severe bronchiolitis with hospitalization, irrespective of viral agent.[478] Previous exploratory studies showed similar results.[479] Birth cohort studies have shown these changes in infant lung function at birth are associated with the risk of

wheezing illnesses through school age.[479-488] The association with wheeze beyond school age and adolescence is less consistent.[486,489-491] Further, both very premature and late born preterm infants have pulmonary structural abnormalities that place them at high risk for respiratory morbidity including bronchiolitis, and also airway obstruction, and increased bronchial responsiveness from infancy through adulthood.[254,492-495] These observations are compatible with the hypothesis that diminished airway size and possibly airway tone shortly after birth are shared host factors for bronchiolitis and asthma.

Third, immature innate and adaptive immune responses may play an important role in susceptibility to bronchiolitis. Studies have found that cytokine responses in cord blood predict the severity of later RSV infection, and cord blood transcripts of genes involved in innate and adaptive immune responses may also determine the severity of disease.[186,496,497] Epidemiological data suggest that interferon type II responses during the first months of life strongly predict the subsequent development of wheezing illnesses, presumably due to viruses, during the first year of life.[498] Conversely, high concentrations of amniotic fluid IL-8 and tumor necrosis factor-α are associated with low risk of RSV bronchiolitis in healthy term infants, leading authors to hypothesize that direct exposure of fetal lungs to pro-inflammatory signals induces local protection against viral infection during infancy.[286] Vitamin D deficiency identified in cord blood of healthy neonates has also been found to be associated with increased risk of RSV LRTI in the first year of life, likely through a complex interaction depending on genetic background, environmental exposure and influencing the developing neonatal immune system.[215,217,307]

On the contrary, a body of evidence also supports the role of viral bronchiolitis in contributing to later pulmonary sequelae through its action during a critical period of lung development. A dose–response relationship between bronchiolitis severity (as defined by inpatient, ED, and outpatient clinic cases) and increased odds of early childhood asthma and asthma-specific morbidity was found using data from the Tennessee Medicaid database.[499] This may be one possible explanation why some population-based studies including milder cases of bronchiolitis show weaker associations with wheezing and asthma than studies restricted to hospitalized cases.

Further evidence stems from the analysis of outcomes of children that were given palivizumab to prevent RSV bronchiolitis. Observational studies found that the use of palivizumab in premature infants with no CLD led to a significant reduction in wheezing episodes compared with control premature infants who did not receive prophylaxis.[500,501] A recent randomized trial in otherwise healthy late preterm infants confirmed that treatment with palivizumab resulted in a significant reduction in wheezing days during the first year of life, even after the end of treatment.[318] This provided the first human experimental proof of concept implicating RSV infection as an important mechanism of recurrent wheeze during the first year of life.

Finally, experimental evidence on animal models has shown a direct effect of RSV on lung hyper-reactivity and chronic airway inflammation, including an increase in susceptibility to allergic sensitization.[502] Clinical data from one of the oldest cohorts of children hospitalized for RSV bronchiolitis by Sigurs et al supports this association. Findings from this cohort have shown an increased risk of wheezing and asthma until adulthood compared with age-matched controls.[503-508] Moreover, allergic rhino-conjunctivitis and sensitization to perennial allergens were also found to be increased (43% vs 17%; and 41% vs 14%, respectively). A persistent/ relapsing wheeze pattern throughout childhood and adolescence was associated with early allergic sensitization and predominated in the RSV cohort compared with controls. The pattern of airway obstruction was more pronounced in these subjects and tracked across ages. This strongly suggests an important interaction between RSV infection and early allergic sensitization.

A middle ground between reconciling both hypotheses is the existence of a bidirectional link between predisposing factors, viral bronchiolitis and asthma, in a complex causal pathway that involves interaction between different factors and mediators.[458] One example is the role of specific viral agent. There is now a consistent body of evidence highlighting bronchiolitis induced by RV (alone or in co-infection) as a stronger predictive factor for recurrent wheezing at preschool and school age when compared to RSV infection.[116,119,157,510-513] Long-term follow-up of the first molecular based viral isolation studies suggests this differential effect of RV bronchiolitis on risk of asthma continues throughout adolescence and possibly into adulthood.[514] RVs have the ability to invade lower airways and escape immunity,

they may promote exaggerated inflammatory responses towards further stimuli, such as allergens, and lead to enhanced airway responsiveness, possibly promoting the development of asthmatic features.[116,157,513] Recurrent RV infections and associated inflammatory and remodeling processes during this time may thus interfere and disrupt normal processes of lung growth. In parallel, children hospitalized with RV bronchiolitis tend to be older, have more atopic risk factors or characteristics (i.e. eczema, allergic sensitization, and parental asthma), and are more likely to have wheezed previously.[93,95,510,515] Thus the connection with subsequent asthma could be explained by an increased susceptibility to RV bronchiolitis among children with an atopic background, in whom the first episode of RV-related wheezing could be the first manifestation of atopic asthma. Conversely, evidence is conflicting as to whether a family history of atopy or asthma is a risk factor for RSV hospitalization. Further, RSV prophylaxis with palivizumab decreases by 80% the relative risk of preschool recurrent wheezing in non-atopic children, but does not have any effect in infants with an atopic family history.[500] Many uncertainties remain, however, including whether this effect will endure regarding later wheezing and asthma diagnosis, how will it affect other domains of asthma (e.g. airway obstruction, bronchial hyper-reactivity, airway inflammation and allergic sensitization). The epidemiological and experimental evidence presented above reflects how asthma results from complex and differential interactions between genetic background, immune responses to allergens and respiratory tract viruses.

Importantly, the discussion on the long-term effects of bronchiolitis per se can hardly be dissociated from other concurrent causal factors of recurrent wheezing and asthma, which are viewed today as complex labels encompassing different traits and phenotypes with likely differing causes, manifestations, treatment responses and natural histories at different ages.[462,516,517] Gene-environment interactions are key to asthma inception, progression and exacerbation, and different factors may contribute as inducers, triggers and modulators.[518] While viral bronchiolitis may be a first step in a “viral march” towards asthma, other determinants and exposures such as airway microbiome diversity, allergic sensitization and exposure, smoking, pollution, obesity, diet and social factors play a role, often interacting between each other and being modulated by genetic background, e.g. presence or absence of family atopy.[7,487,519] The observation that

lung and immune function at birth may predispose to the subsequent development of asthma is not incompatible with the existence of acquired mechanisms for the deficits in lung function and increased bronchial responsiveness observed in asthma. Studies performed with biopsies in wheezing young children suggest that the early preschool years (up to two years) are a developmental window of opportunity for the establishment of deficits in lung function, airway inflammation and remodeling in asthma.[520-523] Interactions between gene and environmental exposures are complex, as seen with the recently explored interactions between 17q21 genotype variants, RV wheezing illness, and tobacco exposure in increasing early wheezing and later asthma.[524-526] Thus, predicting future asthma remains elusive, as there are no simple, valid and universal discriminative and prognostic tools for children with bronchiolitis or recurrent wheezing.[527] Attempts at preventative treatments to reduce post-bronchiolitis wheezing and asthma have also failed.[528-530]

Whether bronchiolitis is also, or rather, a first step or an indicator towards later Chronic Obstructive Pulmonary Disease (COPD) is now the focus of much research. There is a growing body of evidence to support the hypothesis that COPD has its origins in early life.[236,487,531-534] Antenatal factors acting during critical or sensitive periods in early life may result in developmental adaptations that will produce permanent structural, physiological and epigenetic changes with lifetime consequences.[535-537] These include for example tobacco smoke exposure, intrauterine growth, and other maternal and fetal factors, acting on diverse genetic background such as genes related to lung development or adrenergic receptors.[538] Further, cohort studies of infants recruited in the 1980s are now able to demonstrate tracking of lung function from early infancy into adolescence and early adulthood.[52,486,489,491] Thus, even small lung function deficits result in premature airflow obstruction with lung aging. Postnatal factors such as bronchiolitis and other respiratory infections, tobacco smoke exposure and pollution may magnify the accelerated decline in lung function.[538] Since airway function is very likely established by school age, the pre-school years represent a critical time for lung injury and chronic airway obstruction.

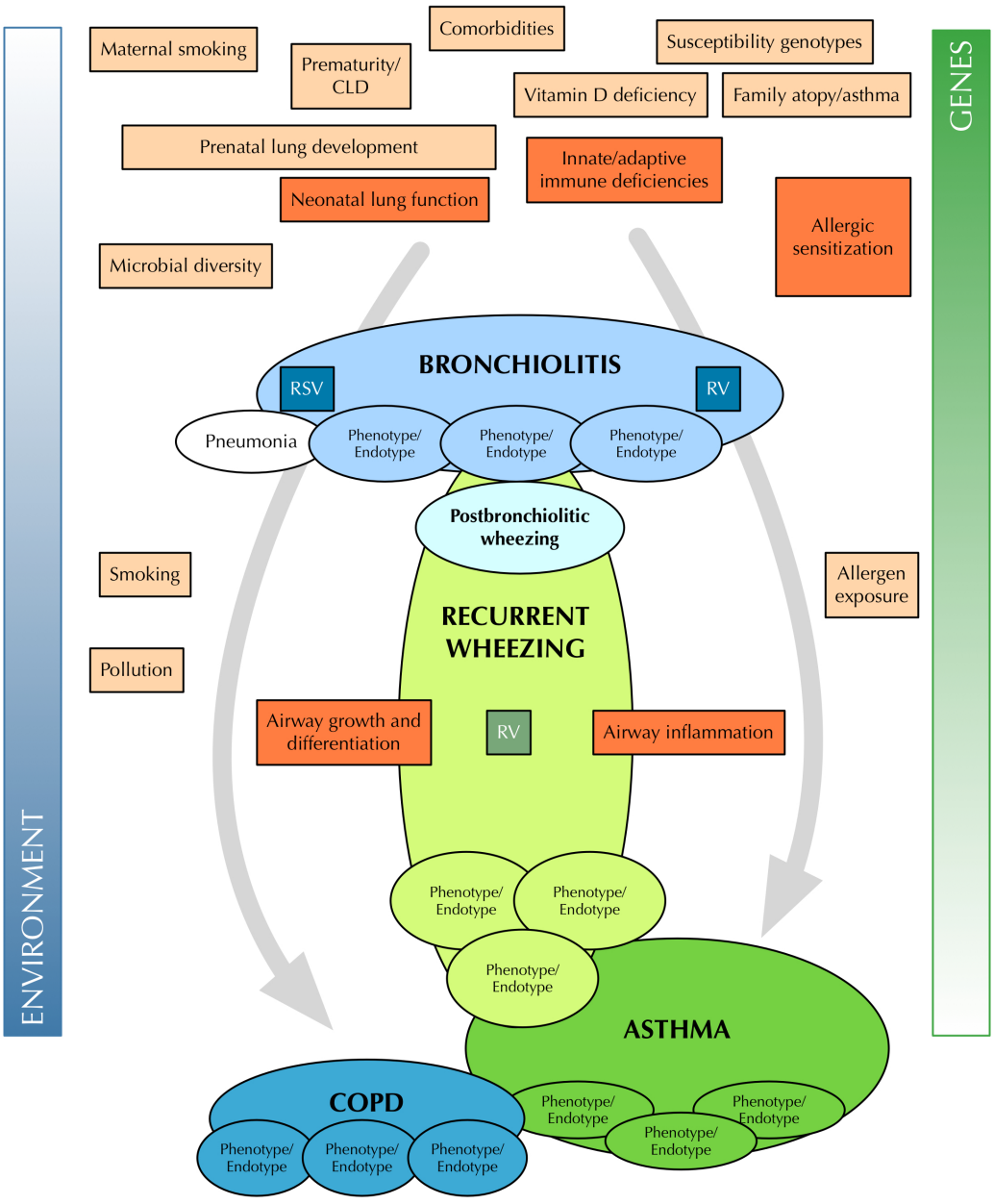


Figure 1.5. Antenatal and postnatal factors in the trajectories from bronchiolitis and recurrent wheezing to asthma and Chronic Obstructive Lung Disease (COPD) (RV: rhinovirus; RSV: respiratory syncytial virus; CLD: Chronic Lung Disease)

1.2 THE DILEMMAS AND LIMITATIONS OF INTERVENTION RESEARCH IN BRONCHIOLITIS

In the first section of the introduction, we have presented an overview of relevant epidemiological, clinical and pathophysiological findings in bronchiolitis, as well as its complex association with long-term respiratory morbidity. Here we focus on the main topics of this thesis: the uncertainties of current evidence on widely used treatment approaches, with emphasis on bronchodilators and corticosteroids, and how this evidence is limited by shortcomings in key areas of clinical trial design, namely disease definition and outcome selection and measurement. We will provide a rationale and backdrop for each question of the thesis partly based on findings present in the first section, and how new methodological developments in evidence synthesis, science of outcomes and measurement, and definition and clustering of disease may help to address them.

GAPS IN EVIDENCE AND PRACTICE IN BRONCHIOLITIS TREATMENT

Therapeutic management of bronchiolitis is an ever-controversial topic in pediatrics.[27,539-541] Treatment options that are reportedly used and/or have been tested include a wide range of pharmacological and supportive interventions.[10] The former include different drug groups, mostly corticosteroids, bronchodilators (β-2 agonists, anticholinergic and adrenergic agents), antibiotics, and hypertonic saline. Other pharmacological therapies rarely used and/or tested include antiviral agents, DNase, montelukast, theophyllines and diuretics. Supportive treatments include feeding and fluids, steam inhalation, chest physiotherapy, and different types of respiratory/airways support, i.e. low or high-flow oxygen therapy (which may be heated and humidified), heliox, continuous positive airway pressure, and intubation with mechanical ventilation. The rationale for use of these treatments varies, but most target proven or hypothesized downstream pathological changes in bronchiolitis, i.e. submucosal edema, bronchiolar and peribronchiolar inflammation, increased mucus secretion, bronchospasm, as well as their physiological consequences, i.e. small airways obstruction, hypoxemia and

respiratory failure. Target populations for these interventions vary, e.g. in terms of timing and severity thresholds for use, as do the levels of care in which they can be implemented also vary. Some of the treatments have also been considered for postbronchiolitis symptoms and/or prevention of recurrent wheezing.[528,542]

Importantly, most specific interventions have failed to show consistent and relevant treatment effects, with absence of clear evidence for any single treatment approach. Nearly 50 years ago Reynolds and Cook wrote that "oxygen therapy is vitally important in bronchiolitis and there is little convincing evidence that any other therapy is consistently or even occasionally useful".[30] During the 1970s, the concept of minimal handling was adopted from neonatal intensive care to the care of infants and small children with acute respiratory distress, emphasizing minimal disturbance and restriction to essential procedures.[543] As of today, no routine treatment is yet recommended by most evidence-based clinical practice guidelines worldwide, and the mainstays of treatment remain oxygen, fluids, and if necessary, respiratory support.[36,393,394] Bronchiolitis is now the focus of promising drug and biologic efforts in their initial stages of clinical development, with most focusing on viral agents as treatment targets (e.g. antiviral RSV drugs).[35,544-546]

There is substantial variation in the use of different therapies for bronchiolitis around the world, as shown in results from practice surveys, retrospective audits and prospective studies. Use of specific pharmacological treatments has been shown to vary at a national, regional and local level. Data obtained from the 2001 to 2009 National Hospital Ambulatory Medical Care Survey in the US showed 53% children below two years of age presenting at an ED with bronchiolitis received short-acting β-agonists, 33% received antibiotics, and 20% received systemic corticosteroids.[547] A prospective cohort study in seven Canadian pediatric emergency departments found 73% of children were treated with bronchodilators, with a significant variation between hospitals in their rate of bronchodilator use (range 59 to 100%) and in the type of bronchodilator used (salbutamol vs adrenaline).[8] Studies from European EDs also suggest frequent and variable use of pharmacotherapy, while some countries such as Australia and New Zealand seem to have lower use of any treatments.[9,44,401,548-555] In inpatients, various North American and European studies have also shown considerable use and site-to-site variation for both pharmacological and supportive treatments.[9,44,551,554-559] Our

nationwide practice survey in Portugal (the ABBA study, part of which is included in this thesis) highlighted differences in management between pediatricians and general practitioners (Figure 1.6), while North American studies have shown differences between general and pediatric EDs.[86,404]

There are many possible motives underlying clinical practice variation on treatment.[560] Table 1.2 present possible reasons for treatment variation in bronchiolitis. Prescribing is a complex task that requires interpretation of evidence in light of individual patient factors, within a context of personal, organizational and systemic factors.[561] The World Health Organization's (WHO) model of rational prescribing described a logical approach that includes making a diagnosis, estimating prognosis, establishing the goals of therapy, selecting the most

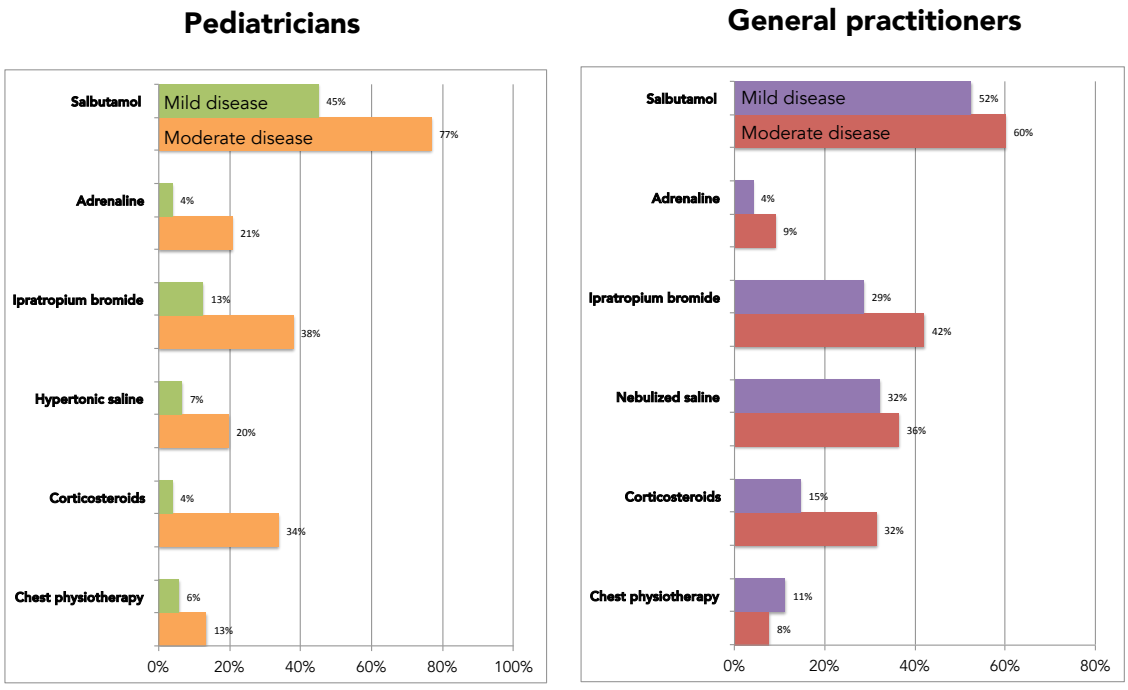


Figure 1.6. Treatment options chosen by pediatricians and general practitioners according to bronchiolitis severity (results from a nationwide electronic survey in Portugal) (from: Fernandes, personal communication)

appropriate treatment and monitoring the effects of the treatment.[562] In bronchiolitis, much research has focused on the concept of unwarranted treatment

variation, i.e. that which is explained not by population differences or health determinants, but by the quality, appropriateness, and efficiency of health care.[563] With most evidence-based guidelines recommending against routine use of most treatments, there seems to be a gap between evidence and practice. Knowledge translation strategies aim to bridge that gap.[564-566] Thus, many studies have evaluated the implementation of clinical practice guidelines and care pathways in reducing unwarranted treatment use.[86,407,551,556,567-572] Despite partial success, however, persistent variation in treatment choices remains. Part of it may relate to barriers in evidence uptake by physicians and health systems. But it extends to the source, quality and interpretability of evidence itself, which are major problems in this field.

Table 1.2: Motives for practice variation in bronchiolitis treatment

Existing evidence	Implementation of evidence
Gaps in current evidence (e.g. lack of comparative effectiveness data)	Diagnostic overlap (e.g. acute wheezing, pneumonia)
Heterogeneity in trial results	Local protocols and experience (e.g. choice of bronchodilator)
	Physician prescribing profile (i.e. urge to act vs "primum non nocere")
	Parental pressure
Both evidence generation and implementation	
Heterogeneous disease definition	
Importance given to a putative early asthma phenotype	

Controversies on the efficacy and safety of older interventions (e.g. bronchodilators and corticosteroids) have persisted for years, while new or revisited treatments (e.g. hypertonic saline, heated humidified high-flow oxygen therapy) are usually championed by some but soon challenged by conflicting evidence. Many medications and devices used in bronchiolitis have not been adequately tested, which hampers rational prescribing and management. Some of the motives for this are common to other pediatric conditions and relate to the paucity and hurdles of pediatric drug research, with frequent off-label and unlicensed use of medicines.[573-575] Many drugs used in bronchiolitis were licensed before rigorous efficacy and safety approval plans were required by regulators, leading to wide disparities in the

scrutiny with which evidence is assessed when compared to newer drugs.[576] Further, requirements on medical device research are relatively recent, so that respiratory support devices have often been implemented without much empirical evidence on their efficacy, safety and applicability in infants.[577] Other reasons are more specific to the field of bronchiolitis. The overlap between bronchiolitis, wheezing disorders and asthma has repeatedly led to the inadequate extrapolation of drug treatment effects, such as with corticosteroids and bronchodilators, despite the uncertainties regarding bronchiolitis immunopathogenesis.[578-580] Further, there is scarcity of pharmacokinetic and pharmacodynamic data for most inhaled therapies in young infants.[581,582] Also, other factors may confound a putative specific treatment effect, such as the type of aerosol delivery device, the solution in which drugs are diluted, and any co-treatments.

Conflicting results with limited validity may hamper one of the main objectives of practicing evidence-based medicine, i.e. actions informed by the best available evidence, what some have called 'doing the right things'.[583,584] Without clear evidence, different physicians will adopt different approaches, on the basis of their beliefs, training, incentives, and the local practice style. In that case, quality improvement efforts to bridge the evidence-practice gap and ensure that evidence is applied thoroughly, efficiently and reliably (what some have called 'doing things right') will have limited effect.[584] Thus it is important to step back and evaluate the uncertainties of existing evidence itself, how they can be improved and how they relate to shortcomings of current research in this field.

CONTROVERSIES AND MYTHS: CORTICOSTEROIDS AND BRONCHODILATORS IN BRONCHIOLITIS

The case of the two most frequently used groups of treatments, bronchodilators and corticosteroids, highlights the uncertainties of research in bronchiolitis. The first randomized clinical trials in this field testing each of these drugs date from the late 1960s.[27,578,585,586] The drugs were meant to target inflammation and bronchospasm, in analogy of the clear benefits in children with acute asthma. However, findings were heterogeneous and conflicting right from the first studies, with distinct interpretations and recurrent controversies. As the growing body of evidence

created the need to synthesize findings from individual studies, the first systematic reviews and meta-analyses often fueled more discussion.[587-590] Here we present a perspective of the clinical pharmacology of both groups of drugs, and we present a rationale for a new and integrated look at current evidence for individual and combined treatment in bronchiolitis.

Bronchodilators: clinical pharmacology and rationale for use in bronchiolitis

Bronchodilators are central in the treatment of airways disorders. Two major classes of bronchodilators have been used in bronchiolitis, adrenoceptor (AR) agonists and muscarinic receptor (M) antagonists.[591] The most frequent fast- and short-acting agents used and tested in bronchiolitis are adrenaline/epinephrine (non-selective α- and β-AR agonist), salbutamol/albuterol (selective β2-AR agonist), and ipratropium bromide (non-selective M receptor antagonist). These agents are mostly administered by inhalation to allow delivery of the drug on the airway mucosa, optimizing their bronchodilator effect and reducing cardiopulmonary side effects. Aerosol generating devices used include mostly nebulizers and pressurized metered dose inhalers with holding chambers.

Bronchodilators work mostly through their direct receptor-mediated relaxation effect on airway smooth muscle (ASM) cells, although other pharmacodynamic targets may be of relevance in bronchiolitis. Their effect on M receptors and ARs relies on the location of these receptors and subtypes expressed. Airway tone is regulated by both the parasympathetic and sympathetic nervous systems, although non-cholinergic and non-adrenergic autonomic neural controls of ASM exist.[592] The parasympathetic system provides the dominant control of smooth muscle tone via efferent cholinergic autonomic nerves. Branches of the vagus nerve travel along the airways and synapse at peribronchial ganglia, from which short post-ganglionic parasympathetic cholinergic fibers innervate ASM, airway glands and microvasculature. Targets of non-selective M receptor antagonists include M3 receptors, located predominantly in central airway ASM cells and submucosal glands, which are key mediators of smooth muscle contraction and mucus secretion .[593-596] Other targets include M1 receptors which facilitate cholinergic transmission, and M2 receptors that provide feedback inhibition. The sympathetic nervous system provides direct innervation of airway vasculature but not ASM, although hormonal catecholamines play a role in regulating the airway tone. Autoradiographic

mapping and in situ hybridization studies show both β1- and β2-ARs are present throughout the lung.[593,596,597] β2-AR agonists act directly on the ASM, with greater distribution of receptors in small rather than large airways. β2-ARs also reduce the cholinergic component of bronchoconstriction, and may mediate effects through other cell types, including bronchial epithelium, submucosal glands and type I pneumocytes (increased mucociliary clearance, possibly increased fluid clearance), type II pneumocytes (stimulation of surfactant secretion), pulmonary and bronchial vascular smooth muscle (vasodilation, possibly reduced permeability), many pro-inflammatory and immune cells (anti-inflammatory effects).[591,598] The consistency of these effects in vitro and in vivo and their translation into relevant beneficial clinical effects in various airways disorders are controversial (e.g. fluid clearance in acute lung injury, or anti-inflammatory effects).[599] β1-ARs in the lung are confined to glands and alveoli and their role is less clear. α-ARs mediate vasoconstriction of pre-capillary arterioles in the airway vasculature, decreasing capillary hydrostatic pressure and leading to fluid resorption and improvement in airway edema. M receptors and α- and β-ARs have wide systemic tissue distribution, which mediate unwanted anticholinergic or adrenergic effects. While the complete nature of interactions between parasympathetic and sympathetic nervous systems is not fully understood, in theory combining β2-AR agonists and M receptor antagonists is pharmacologically reasonable and may maximize the bronchodilator response.[591]

Adrenaline was isolated in 1901 and for years widely used for the treatment of acute asthma, pending later introduction of more selective β-AR agonists with less systemic adverse effects.[591] Both drug names adrenaline and epinephrine have been used in different countries and by different conventions, with discussion as to which is appropriate.[600] Adrenaline was first used parenterally, but multiple α- and β-AR systemic adverse effects led to preference for inhaled use in most airway disorders. The onset of bronchodilation using inhaled adrenaline is very quick (one minute), and the drug has a short half-life, with a duration of action less than two hours.[591] Serious adverse effects of inhaled adrenaline, including β1-AR mediated cardiotoxicity, are likely rare in healthy children.[601] Given its short duration of action, symptoms may quickly return to baseline leading to what is sometimes referred to as the "rebound phenomenon". Racemic epinephrine, which is a 1:1

mixture of the D- and L-isomers, was initially thought to produce fewer systemic side effects, although evidence is conflicting.[602]

The modern era of selective β2-AR agonists began with the discovery of salbutamol (called albuterol in the US), first marketed in the late 1960s.[600] Other less used short-acting β2-AR agonists (SABAs) in bronchiolitis include fenoterol and terbutaline. Salbutamol has a high β2:β1-AR selectivity ratio, and approximately equivalent bronchodilator potency than adrenaline. Maximum bronchodilation can be seen within 15 min of inhalation, but weak receptor biding and quick diffusion back into the microcirculation leads to a short duration of action (4 – 6 hours).[600] Adverse effects of SABAs include ventilation/perfusion mismatch, attributed to differential regional vasodilator and bronchodilator effects, restlessness, muscle tremor and hypokalemia at high doses.[603] Despite its widespread use in most wheezing disorders and asthma, there is scarce pharmacokinetic data on use of salbutamol in preschool children, with heterogeneity in dosing criteria.[604,605] A pure R-isomer of salbutamol, levalbuterol, was developed based on possible unwanted effects from the S-enantiomer in the lung. Animal model studies suggested that levalbuterol might have a better anti-inflammatory effect than racemic albuterol in RSV-infected airways, but evidence is controversial.[606-608] Oral or parenteral administration has been seldom used and tested, despite increasing off target adverse effects without improving delivery of salbutamol to the lungs.

Inhaled M acetycholine receptor antagonists have been used as treatments for respiratory diseases for centuries.[591] Some plants are rich in anticholinergic alkaloids such as atropine, a tertiary ammonium compound with multiple systemic side effects due to its considerable systemic absorption and penetration of the blood-brain barrier. Ipratropium bromide is a quaternary ammonium anticholinergic introduced in 1974 that provides local anticholinergic effects to the lung while avoiding the systemic side effects of atropine as a result of its poor absorption. Ipratropium bromide starts to act within 15 to 30 minutes, but maximal bronchodilation may take up to 90 minutes. Its duration of action is approximately 6 to 8 hours, but compared with SABAs, it has a slower onset of action, although probably a longer duration of action. Ipratropium bromide has a wide therapeutic margin, with scarce evidence of adverse anticholinergic effects i.e. hemodynamic

or ocular effects. Data available on the clinical pharmacology of this drug in infants is also limited.[582]

The rationale for using bronchodilators in bronchiolitis is conditional on the assumption that bronchospasm is a relevant aspect of pathogenesis, but evidence is conflicting. On the one hand, some mechanisms of disease studies have shown that M2 receptors are dysfunctional in patients following lower respiratory tract infection, resulting in unopposed M1 and M3 receptor activity, producing excessive bronchoconstriction.[609] Most evidence, however, comes from asthma studies. Also, neuro-immune pathways may be activated by RSV infection, leading to a change in the distribution and reactivity of sensory and motor nerves across the respiratory tract, causing non-specific airway hyper-reactivity during and after the infection.[610] On the other hand, evidence exists that RSV and RV epithelial infection induce a decrease of β2-AR function on ASM cells.[611-614] Most evidence supports mucosal swelling and mucous plugging as key factors contributing to increased airway resistance observed in bronchiolitis, rather than constriction of bronchial smooth muscle.[6] Adrenaline might have a theoretical advantage over SABAs, as α-AR stimulation could improve airway obstruction by inducing arteriolar vasoconstriction in the airway mucosa and thus reducing bronchial mucosal thickness. The stimulation of α-ARs might also avoid ventilation-perfusion mismatch induced by SABA. Interestingly, in an anecdotal report inhaled adrenaline was found to benefit an infant with RSV bronchiolitis who was also receiving β-adrenergic receptor blockade with propranolol.[615]

Proof of concept studies using lung function outcomes have had inconsistent results, showing that responses to bronchodilators in bronchiolitis range from marked improvement to deterioration of lung function.[616-623] Many studies have provided evidence of airway reactivity to bronchoconstrictor or bronchodilator agents in healthy young infants.[624-627] Discrepancies found in bronchiolitis studies may be due to various factors. First, there may be growth- or maturation-induced changes in the mechanical and functional properties of the respiratory system in infants influencing bronchodilator responsiveness, e.g. distribution and activity of β-ARs, anatomically small airways, increased smooth muscle tone, relatively thick airway walls, decreased chest wall recoil and increased chest wall compliance.[233] It should be noted, however, that one study with lung function outcomes found

bronchodilator responsiveness in infants with bronchiolitis was not age-dependent.[628] Further, when existing, airway responses are not necessarily beneficial: bronchodilators may increase airway wall compliance and reduce the flow in collapsible immature airways, with paradoxical bronchoconstriction.[629,630] Second, it would be tempting to think children with bronchiolitis at higher risk of asthma might have increased bronchodilator responsiveness. Interestingly, Sanchez et al found response to bronchodilator in infants with bronchiolitis could be predicted from wheeze characteristics.[622] However, long-term follow-up results suggest that clinical responses to bronchodilators during acute bronchiolitis or initial episodes of wheezing in infancy are not associated with asthma risk factors, nor have they any association with later asthma at school age.[631,632] Finally, β-AR-related genetic polymorphisms, e.g. ADRB2 genotype, might influence bronchodilator responsiveness.[633] Importantly, results from these studies are limited by the current gaps in infant lung function regarding which techniques may be most useful for assessing changes in airway function, how to quantify the airway response or the potential clinical relevance of findings.[634]

Corticosteroids: clinical pharmacology and rationale for use in bronchiolitis

Corticosteroids (also named glucocorticoids) have been used to treat many pediatric respiratory conditions for over half a century, since the isolation and chemical synthesis of corticosterone and later cortisol, by 1950 Nobel Prize winners Kendall, Reichstein and Henchbeing.[579,635] They are a cornerstone of treatment in acute asthma and croup, where efficacy is well established. These potent anti-inflammatory drugs can be administered systemically or by inhalation, alone or combined with other treatments. Each corticosteroid can be described by its anti-inflammatory potency and specific pharmacokinetic properties.[636] There is considerable variation in dose, duration of treatment and type of regimen used in practice. Systemically administered corticosteroids include prednisone/ prednisolone, probably the most frequently used oral corticosteroids and often used as a reference standard, as well as dexamethasone. Typical use of systemic corticosteroids in acute respiratory conditions conditions is a one to five day high-dose regimen.

Corticosteroids diffuse through cell membranes and bind with high affinity to the cytoplasmic glucocorticoid receptor (GR) to form a complex which translocates to

the nucleus.[637] This complex inhibits inflammation through both direct and indirect genomic, as well as non-genomic effects.[579,637,638] The former include: binding to glucocorticoid-response elements (GRE) in the promoter region of steroid-sensitive genes, with trans-activation of anti-inflammatory proteins; interacting with negative GREs to suppress genes; and interacting with co-activator molecules of pro-inflammatory transcription factors, such as nuclear factor-kB, to inhibit the inflammatory genes that are activated by these transcription factors. Most genes other than those that encode inflammatory proteins are not affected, but gene suppression may explain some adverse effects.[638] Non-genomic effects include signaling through membrane-associated receptors and second messengers, post-transcriptional effects and others. Inhibition of recruitment of inflammatory cells into the airway occurs through the suppression of chemotactic mediators and adhesion molecules, and by accelerating apoptosis. The end result of these molecular pathways is a direct inhibitory effects on many inflammatory cells, including eosinophils, T-lymphocytes, mast cells and dendritic cells. Conversely, corticosteroids inhibit neutrophil apoptosis. Epithelial cells may also be a major cellular target, and corticosteroids may reduce airway microvascular leakage, lead to acute airway mucosal vasoconstriction, and decrease mucus production. [638] Not all effects come into play with different doses and durations of treatment. Importantly, most data are from adult and animal studies, and there is a paucity of information on any developmental effects on these mechanisms.

Prednisone is an inactive prodrug that undergoes oxidative metabolism in the liver to prednisolone, the active drug. Dexamethasone has a greater affinity for the GR than prednisolone, and its biologic half-life (i.e., the time required for the systemic effects to decrease by 50%) is longer than that of prednisolone, both factors accounting for its higher potency. Oral absorption of both drugs is rapid and nearly complete. Some of these formulations are available for delivery as aerosol, and inhaled budesonide is also used.

Corticosteroids require a few hours before they produce a clinical response, and the wash out of effects are also prolonged. Pharmacological research has long focused on the adverse effects from long-term systemic use of these drugs, including growth inhibition, bone disease, infections, cardiovascular disease, adrenal failure and neurodevelopment effects.[639] These effects are known to depend on drug (e.g.

specific corticosteroid, duration and dose) and patient factors (e.g. underlying disease, genetic factors). Neonates and younger children may be particularly vulnerable to long-term effects.[640] There is uncertainty, however, regarding the safety of single or repeated short-duration systemic treatments in otherwise healthy children with conditions such as bronchiolitis.[641] Palatability is an important consideration for oral corticosteroid products, as poor taste or non-liquid formulations are frequent, likely to reduce adherence and contribute to vomiting of medication.[642]

Corticosteroids were originally considered for use in bronchiolitis given inflammation is key to pathogenesis. However, mechanistic studies have suggested that these drugs have limited anti-inflammatory effects in this condition. Various motives may contribute to this. First, neutrophilic inflammation is not responsive to corticosteroids, but plays a major role in bronchiolitis.[175] Systemic administration of dexamethasone did not have a consistent effect on tracheal aspirate concentrations of pro-inflammatory cytokines in children with severe RSV disease.[643] Other studies suggest RSV may have a deleterious effect on corticosteroid signaling and repress GR-mediated gene activation.[644,645] Some authors have suggested that corticosteroid treatment response is virus-specific in wheezing children. A trial by Lehtinen and colleagues suggested that children with RV infection responded to prednisolone, as opposed to those with RSV.[646] However, this study was a post hoc analysis and included children with recurrent wheezing; long-term follow-up seems to contradict the initial positive finding.[509,513,646] Studies assessing the use of longer courses of corticosteroids started during the acute phase for the prevention of post-bronchiolitic wheezing have also failed to show a long-term effect.[528-530] Further, there is an ongoing debate regarding their efficacy in acute virus-induced wheezing in preschool children.[647-649]

While the interactive effect of bronchodilators and corticosteroids has been widely known in asthma, both at a clinical and biological level, its use as a putative treatment option in bronchiolitis has only been explored recently. Combination therapy with inhaled corticosteroids and long-acting β2-ARs is key to the management of asthma and also COPD. Several synergistic effects at the molecular level have been described when combining therapies, which support clinically relevant effects for both conditions.[650,651] On the one hand, the β2-AR gene

contains several GRE sequences in its promoter region, and their stimulation by corticosteroids results in an accelerated rate of transcription of the β2-AR gene, with unregulated expression of the receptor. The efficiency of coupling between the β2-ARs and downstream G-proteins may also be modulated by corticosteroids. On the other hand, experimental evidence indicates that long-acting β2-AR agonists can promote and accelerate translocation of the GR complexes from the cytosol to the nucleus. Whether these mechanisms are relevant for acute, short-term use of both bronchodilators and corticosteroids, by different modes of administration (i.e. inhaled vs. systemic) and with different specific agents (i.e. long- vs. short-acting bronchodilators, β2-ARs vs. other bronchodilators) is not known.

Integrating evidence from multiple treatments using evidence synthesis

The purpose of systematic reviews is to collate relevant evidence from individual studies to answer a specific research question.[652] The formal synthesis and integration of research studies may be attributed to the advent of meta-analysis in the early 1970s within the areas of educational and psychological research.[652] Over time numerous approaches to synthesizing research evidence have emerged. Systematic reviews use explicit, systematic methods in an effort to be as comprehensive as possible and to minimize bias in the results and conclusions. The Cochrane Collaboration has been instrumental in developing methods for systematic reviews related to interventions in healthcare.[653] Previous systematic reviews, including Cochrane reviews, have examined the efficacy and safety of various bronchodilators and corticosteroids in bronchiolitis.[587,589,654,655] However, there are four main motives to support a new synthesis effort:

1. to include recent evidence from large clinical trials;
2. to explore the use of combined therapy;
3. to address shortcomings in previous reviews; and
4. to use novel multiple treatment synthesis methods to integrate this evidence.

The two largest randomized clinical trials in this field were recently published. The CanBEST trial included 800 children from Canada, and used a factorial design to examine adrenaline and dexamethasone, alone or combined, compared with placebo.[46] Another trial completed concurrently in the United States compared dexamethasone with placebo in a sample of 600 children.[45] Updating of systematic reviews is a major issue given the inevitable and rapid accumulation of new

research findings. Different systems have been proposed to identify which reviews may be out of date, including time-based periodic updating policies, or surveillance systems adjusted to the progress of research in each specific field.[656] In this case, the review on corticosteroids was withdrawn from the Cochrane database in 2008 due to lack of an update.[655] Further these two large trials add substantially to the evidence and provide a strong signal for further synthesis work.

The CanBEST trial raised new questions regarding combination therapy, by showing a 35% relative reduction on rates of admission to hospital with combined adrenaline and dexamethasone treatment compared with placebo. Combination therapy has not been examined previously at the level of systematic reviews nor placed in the context of other evidence. Examining potential additive (synergistic) or subtractive (antagonistic) effects between treatments such as bronchodilators and corticosteroids at a trial and systematic review level is challenging.[657] At the trial level, the factorial trial design such as the one used by the CanBEST trial may answer these questions. However, this design requires special methodological considerations, particularly when interactions are not the focus of the study and are not anticipated, such was the case.[658] Further, co-interventions that are not the main focus of study, such as use of bronchodilators (or, conversely, use of corticosteroids), are often used at the discretion of the attending physician in both arms parallel trials, which confounds analysis. While there is scarce guidance on how to investigate synergism/antagonism at a systematic review level, the fact that some studies use protocolized treatments provides an added opportunity to compare trial results at a subgroup level.

Limitations of previous systematic reviews in this field include inconsistencies in definitions of disease, interventions studied, selected outcomes and methods used. [10] These differences were found both between reviews of different treatments, but also between reviews focusing on the same group of treatments. For example, two previous systematic reviews that assessed the use of corticosteroids in acute bronchiolitis focused on different populations and interventions: Garrison et al only included inpatient trials and was restricted to systemic corticosteroids, while King et al included children from all settings and all treatments; none excluded children with previous episodes of wheezing, nor used an age limit.[587,589] Primary outcomes of each review also differed, even among reviews that focused on the same specific

population or setting. Finally, approaches to data analysis also varied, particularly in non-Cochrane systematic reviews. For example, some reviews either did not pool quantitative results or did so by analyzing modified composite outcomes.[587,589] None of the reviews used standardized updated instruments to assess risk of bias, such as the Cochrane 'Risk of bias' assessment tool, or to grade the quality of evidence, such as the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.[659,660] Differences between methods have largely contributed to controversies in the interpretation of review findings. A comprehensive review with a broad scope that would include both bronchodilators and corticosteroids using a coherent standardized approach and current methodological guidance would allow to collate relevant evidence from individual studies to describe the relative benefits and harms of these interventions.

A final motive to conduct a new systematic review in this field relates to emerging analytic methods in synthesis research, including network meta-analyses (NMAs).[661,662] These methods help to address some of the aforementioned challenges and limitations of previous analyses, while providing new information on comparative effectiveness of interventions. A limitation of previous individual systematic reviews is that they are narrow in scope, focusing on direct pairwise comparisons and excluding competing interventions. For example, no previous review compared evidence between different bronchodilators, or between bronchodilators and corticosteroids. When many competing treatments exist, meta-analyses lack formal comparisons across different interventions that are critical for informed decision making, e.g. to determine which is the "best" treatment to use in bronchiolitis. The Cochrane Collaboration introduced a new form of reviews, the "overviews of reviews", which summarizes evidence for the relative effectiveness of several interventions for the same condition. The Cochrane Child Health Field has contributed to refining the methods of overviews and has been a leader in producing them.[663] We contributed to one such overview focused on interventions for bronchiolitis, bringing together 11 individual previous Cochrane systematic reviews.[10] However, authors of overviews are dependent on the decisions and methods used within the relevant systematic reviews. Further, overviews of reviews do not usually integrate this evidence coherently and quantitatively, making it difficult to judge which treatment should be used.[662] In the bronchiolitis overview, readers might inappropriately make their own indirect comparisons between

treatments (e.g. comparing treatment effect estimate in admission rates from salbutamol or adrenaline vs. placebo), which could result in misleading conclusions. One solution is to conduct 'comparative effectiveness reviews' or 'comparing multiple interventions reviews', which provide a coherent evidence base that reflects the network of comparisons that arises when collating studies involving different subsets of competing treatments.[662] These reviews have an optional quantitative part, which is variably known as NMAs, multiple-treatments meta-analysis, or mixed-treatment comparison.[664] The terms are often used interchangeably, and they refer to the same framework that combines direct and indirect information across a network of randomized trials to infer about the relative effectiveness of multiple interventions. The idea of indirect comparison, which underlines the methods, is a simple one: we can compare treatment A to treatment B via a common comparator C, by statistically combining the information from A versus C (AC) and B versus C (BC) studies (Figure 1.7).[665] An NMA typically uses a Bayesian network model to compare all interventions simultaneously. While a network analysis in a frequentist framework is possible, at the present time there is much more methodology and support available for conducting these analyses in a Bayesian framework.[664] NMAs are appealing because they can provide evidence about the relative effectiveness across a range of interventions, for instance they can provide a ranking of interventions which appears to be the most effective or can compare a given intervention with all other potential interventions. Incorporating both direct and indirect evidence can also provide stronger inferences about relative treatment effects, even when direct head-to-head comparisons are not available. The ability to compare treatments that have not been directly compared in any trial could allow a better evaluation of combination therapy. Nevertheless, several assumptions need to be met for the results of an NMA to be valid.[666,667] In particular, conceptual and statistical heterogeneity (i.e. disagreement between estimates within the same comparison), and incoherence (i.e. differences between direct and indirect evidence in the network) should be assessed.

In summary, we have presented motives that support the need for conducting a comprehensive comparative effectiveness review, with network meta-analysis, focusing on bronchodilators and corticosteroid use in bronchiolitis (Chapter 2). A further advantage of such effort was to have offer a panorama of current clinical trial research in this field. In particular, NMAs include the visualization of the

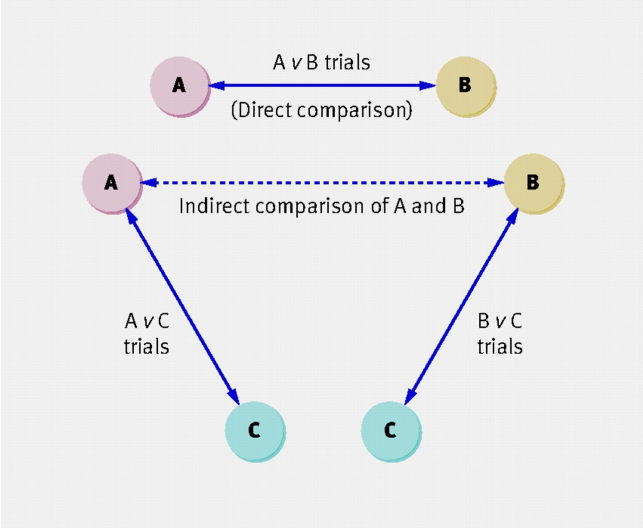


Figure 1.7. Trial network with direct and indirect comparisons. Each trial network consists of three sets of independent trials: one set for direct comparison of A versus B and two sets for adjusted indirect comparison of A versus B with C as common comparator (From: Song, with permissions)

network geometry of comparisons performed in included trials, which allows one to understand how much evidence exists for each treatment, whether some types of comparisons have been avoided, and whether particular patterns exist in the choices of comparators. Further, such a comprehensive review would allow to better study another topic of this thesis: the heterogeneous choice of outcome measures.

OUTCOMES AND MEASUREMENT INSTRUMENTS IN BRONCHIOLITIS

Selection of appropriate primary and secondary outcomes is essential for study design, as ultimately, any study is only as credible as its endpoints.[668] To be useful, clinical trials that evaluate benefits and harms of interventions must measure outcomes of relevance to stakeholders.[669] These include practitioners and patients or their proxies who make shared decisions about treatment options, regulatory authorities that assess and monitor approvals of drugs and devices, as well as health care funders and policy makers. Further, instruments used to measures these

outcomes must be scientifically sound and have measurement properties that are fit for this evaluative purpose.[670,671]

Inconsistent selection, measurement, and reporting of outcomes in clinical trials hampers the scientific, ethical and economic significance of RCTs.[672] Three main problems may arise.[673] First, outcomes may not consistently reflect endpoints that are meaningful for patients or other stakeholders. For example, one study found that outcomes in clinical trials of children with asthma tend to focus on how interventions affect short-term symptoms, measures of lung function, and acute exacerbations of illness, with longer-term outcomes, quality of life, and functional status being measured much less frequently. In clinical trials in neonatology, a reliance on short- term outcomes, rather than longer-term benefits and harms of interventions, has also been shown.[674] Second, inconsistency in measurement domains and instruments is a barrier to compare, contrast, and combine trial findings, further affecting their interpretation. In the 1990s, the potential scale of the problem of multiple outcome measures was highlighted in a comprehensive review of 2000 trials in schizophrenia which were found to have used 640 different rating scales in their assessments of 600 interventions.[675] A recent update of this data found on average, a new instrument to assess schizophrenia had been introduced for every fifth trial.[676] Similar findings have been replicated in other fields. Cochrane reviews usually describe inconsistencies in the outcomes reported in eligible trials and regularly conclude for the need to standardize outcomes.[672,673] In addition, there is great variability in the quality (for example, in reliability and validity) of outcome measurement instruments used and it is not always clear if the best instrument is being used for a given outcome. For example, fewer than 20% of 906 different outcomes measured in breast reconstruction surgery trials were defined or measured with a validated tool.[677] Third, if researchers have measured a particular outcome in a variety of ways, they might not report all of their findings from all of these measures. Outcome reporting bias can ensue, if the results of an analysis are used to choose which outcomes will be reported, e.g by selectively reporting only the most positive or statistically significant results.[678-680]

In recent years there has been a call for the standardization of outcomes in clinical trials.[672,681,682] Concurrently, the GRADE system has suggested that systematic reviewers and guideline panels identify critical outcomes that are important to

patients.[683] Current limitations in outcome selection could be addressed with the development and application of agreed standardized sets of outcomes that have been termed 'core outcome sets'.[681,682] These should be measured and reported, as a minimum, in all relevant clinical trials for a specific condition. Adopting a core outcome set does not imply that a researchers are limited in their choice of primary or other outcome measures of interest, or that these should be restricted to only those outcomes. Rather, the expectation is that, as a minimum, core outcomes will always be collected and reported, which would allow an adequate assessment, comparison and synthesis of treatment effects of interventions between trials. Core outcome sets would foster research and consensus in identifying scientifically sound, clinically relevant and patient-important outcome measures for different conditions and interventions. Importantly, it would enhance the value of evidence synthesis by reducing the risk of outcome reporting bias and ensuring that all trials contribute usable information for end users.

Some historically successful initiatives in standardizing outcomes can be used as examples. One of the earliest examples was an initiative by the WHO in the late 1970s, that led to guidelines on the minimal requirements for data collection in cancer trials. The Outcome Measures in Rheumatology initiative (OMERACT) has pioneered the development of core outcome sets within rheumatology since 1992.[668,681,684] Other groups have been working on core outcome sets or related guidance in specific areas of health care, including the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) for chronic pain trials, Harmonizing Outcome Measures for Eczema (HOME), and TREAT-NMD Neuromuscular Network for neuromuscular disease. [685-687] A recent systematic review found a total of 198 studies on core outcome sets, most commonly in cancer, rheumatology, neurology, heart and circulation, and dentistry and oral health.[688] Interestingly, few studies address the appropriate choice of outcomes for clinical research with children, and in most pediatric specialties no research has been undertaken.[689] The COMET (Core Outcome Measures in Effectiveness Trials) Initiative, launched in January 2010, brings together researchers interested in the development, application and promotion of core outcome sets, derived using rigorous consensus methods, for effectiveness trials.[673,682,690]

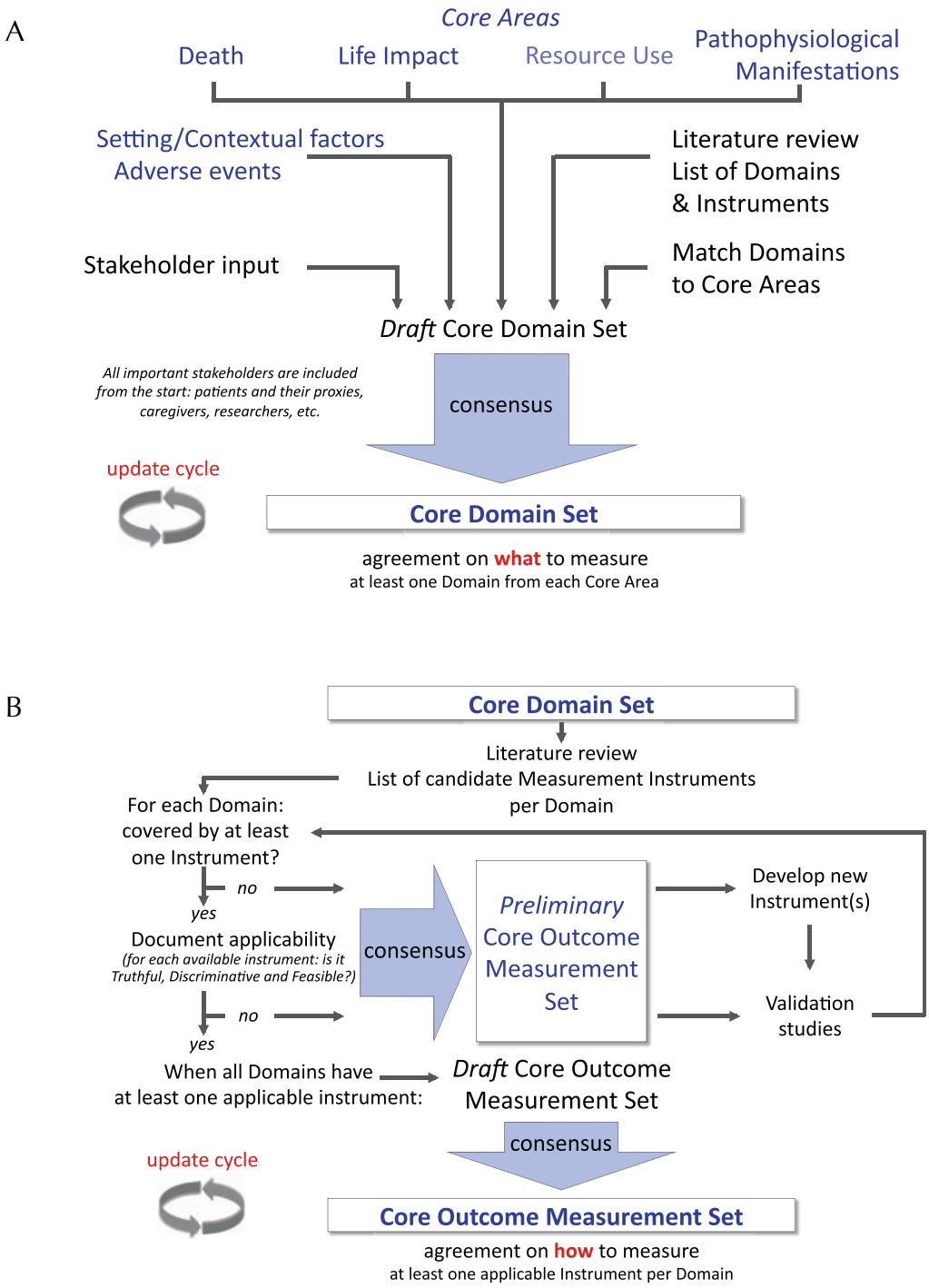


Figure 1.8. Stepwise approach to the development of a Core Domain Set (A) and a Core Outcome Measurement Set (B), as proposed by the OMERACT group (adapted from: Boers, with permissions)

Methods to support the development and implementation of core outcome sets are still evolving.[682,691] Gargon and colleagues have shown how a range of methods have been used; it is uncertain which are most suitable, and there is limited empirical evidence regarding whether different methods lead to similar or different conclusions.[688] Some core outcome sets have been developed through a literature review of outcomes used in trials, given to consideration by an expert panel of health care professionals; these then select relevant domains, often through ranking, and sometimes include suggestions for instruments to measure these domains.[689,692] Problems with this approach include failing to involve all stakeholders such as patients, not explicitly using a conceptual framework for choice of outcomes, and not basing instrument selection on systematic evidence of their measurement properties.[691] Thus, these core sets may overlook important concepts and recommend inadequate measurement tools. Key issues in the development of a core outcome set include: clear definition of its scope, i.e. health condition, population and interventions; identifying existing knowledge comprehensively but efficiently; involving all relevant stakeholders using adequate and feasible consensus methods that allow for methodological rigor and inclusion of a diverse range of opinions; reviewing with feedback and updating; and implementing the core outcome set.[682] Importantly, one must distinguish between potential domains (''what to measure'') and measurement instruments (''how to measure''); and the process to identify these and to reach consensus on which to include in a core set.[691] Table 1.3 presents definitions of key concepts in this field, albeit terminology varies for some of these concepts.

Table 1.3: Definitions of key concepts regarding outcomes and outcome measurement (adapted from Boers et al, Mokkink et al, and Williamson)

Concepts and definitions			
(Sub)Domain	Concept to be measured, a further specification of an aspect of health		
Outcome	Any identified result in a (Sub)Domain arising from exposure to a causal factor or a health intervention; generic word that has been used with different definitions; has often been used interchangeably with "outcome measure" and "endpoint."		
Core Domain/ Outcome Set	For studies of health interventions, the minimum set of Domains and Subdomains necessary to adequately measure all relevant concepts of a specific health condition within a specified setting. Describes what to measure. OMERACT uses the term "Core Domain Set", while the COMET initiative uses the term "Core Outcome Set".		
Measurement instrument	A tool to measure a quality or quantity of a variable, in this context a (Sub)Domain or a contextual factor. The tool can be a single question, a questionnaire, a score obtained through physical examination, a laboratory measurement, a score obtained through observation of an image, and so on.		
Core Outcome Measurement Set	The minimum set of outcome measurement instruments that must be administered in each intervention study of a certain health condition within a specified setting to adequately cover a corresponding Core Domain Set. Describes how to measure.		
Measurement Properties	Refers to the properties of a measurement instrument; the consensus-based COSMIN taxonomy and definitions are shown below.		
Reliability	The degree to which the measurement is free from measurement error; the extent to which scores for patients who have not changed are the same for repeated measurement under several conditions: using different sets of items (internal consistency), over time (test-retest) by different persons on the same occasion (interrater) or by the same persons (i.e., raters or responders) on different occasions (intrarater)		
	Internal consistency	The degree of the interrelatedness among the items	
	Reliability	The proportion of the total variance in the measurements which is because of "true"a differences among patients	
	Measurement error	The systematic and random error of a patient's score that is not attributed to true changes in the construct to be measured	
Validity	The degree to which an instrument measures the construct(s) it purports to measure		
	Content validity	The degree to which the content of an instrument is an adequate reflection of the construct to be measured	
		Face validity	The degree to which (the items of) an instrument indeed looks as though they are an adequate reflection of the construct to be measured
	Construct validity	The degree to which the scores of an instrument are consistent with hypotheses (for instance with regard to internal relationships, relationships to scores of other instruments, or differences between relevant groups) based on the assumption that the instrument validly measures the construct to be measured	
		Structural validity	The degree to which the scores of an instrument are an adequate reflection of the dimensionality of the construct to be measured
		Hypotheses testing	Idem construct validity

Table 1.3: Definitions of key concepts regarding outcomes and outcome measurement (adapted from Boers et al, Mokkink et al, and Williamson)

Concepts and definitions			
		Cross-cultural validity	The degree to which the performance of the items on a translated or culturally adapted instrument are an adequate reflection of the performance of the items of the original version of the instrument
	Criterion validity	The degree to which the scores of an instrument are an adequate reflection of a "gold standard"	
Responsiveness	The ability of an HR-PRO instrument to detect change over time in the construct to be measured		
Interpretability	The degree to which one can assign qualitative meaningdthat is, clinical or commonly understood connotationsdto an instrument's quantitative scores or change in scores.		

The issue of consistency and relevance of outcomes and measurement instruments is not new in bronchiolitis. Authors have commented how the use of different outcome variables, often focused on short-term and surrogate variables of respiratory distress, has limited the interpretation of trial results. [27,693] Further, it is known that many respiratory scales were developed ad hoc, and their measurement properties have not been studied adequately.[694] Systematic reviews and guidelines have repeatedly indicated research in this field as a priority, and recommended the use of outcomes that are relevant to parents, clinicians, and health systems.[695] However, the extent of these gaps in outcome measurement is not known, and little original research has contributed to improve current measurement tools.

What to measure: conceptual frameworks and outcome domains

Outcomes may encompass a spectrum of different aspects of health or health dimensions or domains, each with distinct implications, e.g. biological, clinical, or patient-oriented perspectives. Because of the multidimensional aspects of health, researchers have used a variety of conceptual frameworks for considering the effects of illnesses and measurement of health across a range of outcome domains.[696] A conceptual framework or model is a schematic representation of a theory that acts as a heuristic device to provide a better understanding of a phenomenon (e.g., health or health-related quality of life) by depicting interrelationships among concepts.[671] These frameworks are useful for core outcome set development, by supporting and making explicit a comprehensive and coherent choice of areas/ domains of core set development. A recent scoping review found five such conceptual frameworks: the WHO tripartite definition of health ("a state of

complete physical, mental and social well-being and not merely the absence of disease or infirmity"); the 5 Ds (discomfort, disability, drug toxicity, dollar cost, and death), the International Classification of Functioning, Disability and Health (ICF; based on functioning at the level of body or body part, the whole person, and the whole person in a social context); the Patient-Reported Outcomes Measurement Information System (PROMIS; based on physical health, mental health, and social health); and Porter's Outcome Hierarchy (based on health status achieved or retained, process of recovery, sustainability of health).[692] Health-related quality of life frameworks also exist, with the most frequently used being the Wilson and Cleary model, and its modification by Ferrans.[697,698] Some of these frameworks or their adaptations have been used in previous core outcome set development studies, while other researchers have failed to use a conceptual model.[692] However, differing conceptualizations limit the ability to have a coherent body of evidence to guide further core outcome set development.

The OMERACT initiative has developed and updated a distinctive and comprehensive conceptual framework and a recommended process to develop core outcome measurement sets for rheumatology, which is likely to be useful as a template in other areas of health care.[691] This framework comprises core areas that should encompass the complete content of what is measurable in a trial: three areas that describe the "Impact of Health Conditions," specifically Death, Life Impact, and Resource Use; and a fourth area that describes "Pathophysiological Manifestations" (Figure 1.8). These areas may include various concepts of interest, i.e. domains or subdomains. A suggested stepwise approach to the development of a Core Domain set, i.e. "what to measure", is shown in Figure 1.8. It implies deciding on the setting or scope, contextual factors and adverse events, followed by a literature search to document all domains used to date, while initiating stakeholder consultation to determine what is essential to measure. Work by the COMET group has shown that a variety of methods have been used to achieve consensus within and between stakeholder groups on core outcome sets, including semi-structured discussion, unstructured group discussion, the Delphi technique, expert panel meetings, surveys and Nominal Group technique.[682] Researchers should consider the potential impact of group composition, questioning technique, the information participants receive, whether or not responses are anonymous,

interactions between group participants, the medium of the interaction, attrition bias, analyses, and the way in which consensus is reached.

A review of previous trials or systematic reviews can provide evidence of need for a core outcome set in a certain area, and also identify a potential list of outcomes. Building partially on the comprehensive comparative effectiveness review planned for Chapter 2, as well as on a recent overview of reviews, Chapter 3 will address preliminary work in identifying primary and secondary outcomes selected and reported in current clinical trials of a range of interventions. Further, there is scarce evidence on which health domains are clinically relevant and patient-important in bronchiolitis. As one the most common acute diseases in childhood that encompasses a large spectrum of severity, it is likely that stakeholder perspectives vary by a number of factors. Bronchiolitis is at a crossroads of different levels of care (i.e. primary care, ED, inpatient, intensive care) and different physician specialties (general practitioners, general pediatricians, ED specialists, hospitalists, pediatric pulmonologists, intensivists). There is wide practice variation, and organizational differences and subjective clinical decision-making may influence main outcomes such as hospital admission rates and length of stay. Preexisting comorbidities, demographics, family history and disease severity might also affect parents' and caretakers' perspectives. Moreover, it is an acute and dynamic condition, but may recur and have long-term influences. These are challenges when integrating multiple perspectives in a core outcome set. A first step described in Chapter 4 includes a large scale survey of physicians in different settings that addresses (among other topics) the utilization of outcome measures.

How to measure: measurement properties of measurement instruments

Once a core outcome set or core domain set is defined, it is important to achieve consensus on how these outcome domains should be measured.[682,691] When selecting health measurement instruments for use in clinical trials, a number of aspects need to be considered a priori, such as the constructs to be measured, the target population, and the goals of the intervention.[671] It is imperative that these instruments cover the adequate concepts, and that their measurement properties (i.e. their validity, reliability, and responsiveness), are adequate and applicable for a purpose of evaluation, both in the population included in the trial and in the setting in which the trial is conducted. This issue is particularly relevant in pediatrics, as

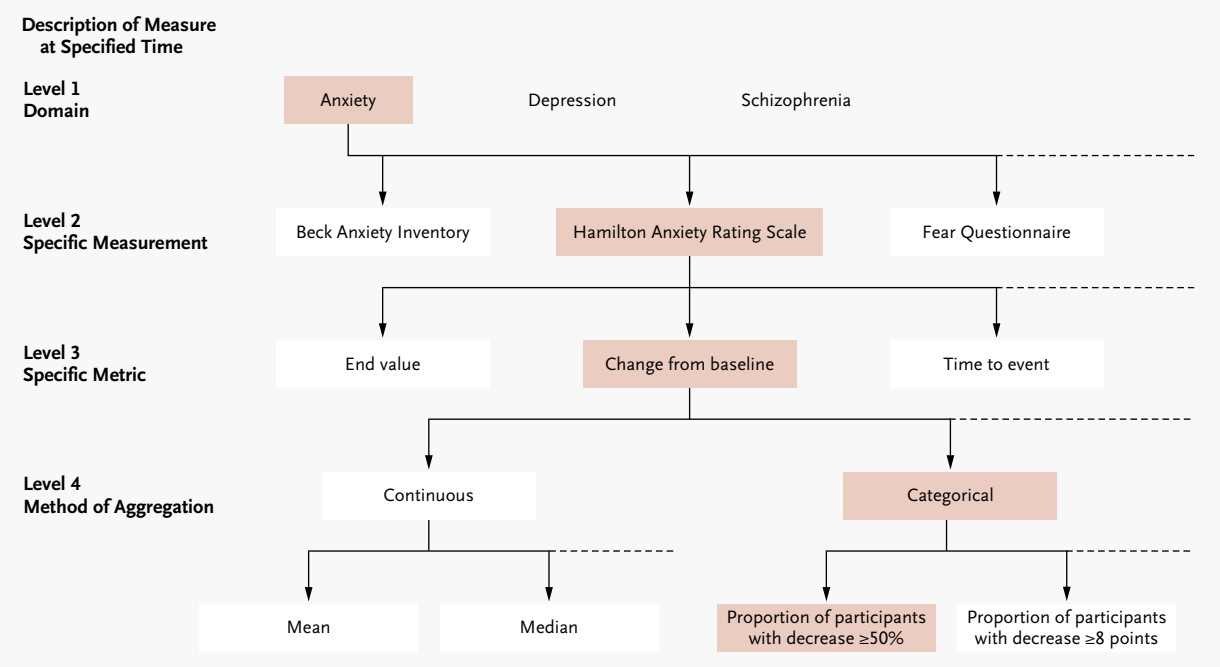


Figure 1.9. An Example of the Four Levels of Specification in Reporting Outcome Measures (Reproduced with permission from Zarin et al, Copyright Massachusetts Medical Society.)

instruments may be used based on the extrapolation of data from adults without proper validation and feasibility in children.[699] Further, the metrics and type of analysis chosen are also relevant (Figure 1.9).[700]

Measurement in medicine is hampered by a lack of evidence on which are the best instruments. Absence of consensus on taxonomy, terminology, and definitions has led to confusion about which measurement properties are relevant, which concepts they represent, and how they should be evaluated.[701] Integration between different perspectives on measurement by scientific disciplines such as psychometrics and clinimetrics, has been the subject of debate.[702,703] The Consensus-based Standards for the selection of health Measurement Instruments (COSMIN) initiative developed through consensus a taxonomy of measurement properties and their relationships relevant for evaluating health instruments, which is shown in Figure 1.10 and Table 1.3.[701] These include internal consistency, reliability, measurement error, content validity (including face validity), construct validity (subdivided into structural validity, hypotheses testing, and cross–cultural validity), criterion validity, and responsiveness. Design requirements and preferred statistical methods were also

Figure 1.10. COSMIN taxonomy of relationships of measurement properties. Abbreviations: COSMIN, COnsensus-based Standards for the selection of health Measurement INstruments; HR-PRO, health related-patient reported outcome. (based on COSMIN handbook, with permissions)

agreed upon. The results of the consensus were used to construct the COSMIN checklist, which can be used for systematic reviews of measurement properties to evaluate the methodological quality of the included studies on measurement properties, to assess to assess the quality of a measurement instrument when used in combination with criteria for good measurement properties, to design and report studies on measurement properties, and to identify the need for further research.[704,705] The original OMERACT Filter published in 1998 is another approach used to determine applicability of a measurement instrument in a setting.[684] It summarizes

key instrument properties in three plain language words, namely Truth, Discrimination, and Feasibility, with feasibility of measurement as an important consideration.

There is considerable debate and lack of guidance on the criteria for good measurement properties to support measurement instrument selection for clinical trials. This is illustrated when considering a key measurement property, responsiveness, and another important concept, interpretability. Instruments with an evaluative purpose that are meant to be used longitudinally to measure change over time should be responsive. However, many different definitions of responsiveness have been proposed, as well as a number of distinct methods to assess it.[706] Further, while interpretability is not considered a measurement property, it is an important requirement for the suitability of an instrument in research and clinical practice.[701] Multiple methods have been proposed to support the interpretation of change scores or differences, both within or between groups of patients followed over time. In particular, a variety of statistical and anchor-based approaches are available to ascertain the Minimal Important Change (MIC), i.e. the smallest change in score that is perceived as important by patients, clinicians or relevant others.[707-711] However, no consensus exists on which methods are most appropriate, terminology varies (e.g. some authors prefer Minimal Important Difference - MID), and there is some debate as to how the MIC relates to measures of measurement error, e.g the Smallest Detectable Change.[707,709,710,712,713]

Selecting measurement instruments to measure the core domains can start with a literature review that provides a list of available measurement instruments, followed by systematic reviews of their measurement properties evaluating the methodological quality of the studies and the quality of the instruments.[682,691,705] Instruments are then selected based on this assessment, but development of new instruments may be needed. Project 2 of this thesis will provide an overview of commonly used instruments in clinical trials of corticosteroids and bronchodilators in bronchiolitis. Previous reviews have identified the use of various scales to assess respiratory status in bronchiolitis, including the Respiratory Distress Assessment Instrument (RDAI) and the Respiratory Assessment Change Score (RACS). These instruments were first described by Lowell in a trial assessing the effect of adrenaline in wheezing infants.[714] The RDAI is a scale of respiratory distress,

assessing retractions and wheezing, while the RACS is a change score based on change in both RDAI and respiratory rate (Table 3.3, Chapter 3). Despite their frequent use, systematic reviews of asthma or wheezing instruments have found limited evidence regarding the RDAI and RACS measurement properties and their suitability for use as evaluative instruments in clinical trials.[694,715,716] A recent RDAI validation study reported poor construct validity, poor inter-rater reliability and mild responsiveness.[717] The RDAI was used as an outcome measure in the largest clinical trial in this field. Chapter 4 of this thesis will use data from RCT to study the measurement properties of the RDAI and RACS, i.e. validity, reliability and responsiveness, using COSMIN's taxonomy.

DEFINING BRONCHIOLITIS: MOVING FROM WORDS AND LABELS TOWARDS PHENOTYPES

While bronchiolitis is a relatively straightforward clinical diagnosis for most child health practitioners today, no standardized set of diagnostic criteria exist.[2] The debate over the definition of disease has lasted for decades, and its origins can be traced back to the emergence of bronchiolitis as a distinct clinical entity.[19,30] Previously we've explained how consistent clinical descriptions of seasonal epidemics of infants with hallmark findings were decisive in the gradual recognition of the condition during the 1960s. The isolation of RSV and its prominent role as an etiologic agent concurred. However, heterogeneous features of bronchiolitis were identified early on and various authors repeatedly reflected on its implications.[19,30,718-724] Surprisingly, despite the frequency and importance of the condition, no significant progress was made in systematically addressing the lack of clarity on disease definition. Many motives may contribute to the latter, including: subjectivity and variability in the identification and interpretation of cardinal clinical findings; absence of accurate diagnostic tools; diagnostic overlap with conditions such as pneumonia and acute wheezing; different perspectives according to the spectrum of severity, levels of care, settings, specialties and context; perceived or real diagnostic and prognostic implications of having underlying risk factors for severity and asthma; and terminology and nosology issues.

While most existing bronchiolitis definitions highlight how bronchiolitis is a clinical diagnosis, there are important nuances in details of the constellation of demographics, history and physical examination findings. This is highlighted when comparing definitions proposed by landmark opinion papers, current clinical practice guidelines, and inclusion criteria from recent large clinical trials (Table 1.4). Variability is focused on key demographic and clinical items, including age, number of episodes and auscultatory findings. A 2004 systematic review by Viswanathan and colleagues reviewed the case definitions used in bronchiolitis clinical trials and found variability in included symptoms, with authors frequently using a broad "physician-diagnosed" criteria without further details.[695] Authors have highlighted how age limits vary in previous bronchiolitis observational studies.[95,725] When looking at the two largest clinical trials performed in this field, it is also interesting to note that large numbers of infants are screened but a large proportion of exclusions is due to definition-related factors such as having a history of wheezing.[45,46] Further, some studies restrict bronchiolitis based on other factors such as specific viral agents (e.g. RSV), or use heterogeneous thresholds of severity (e.g. hospitalizations, ICU admission). The methods and rationale for these definitions is often not reported, and most authors agree that there is little evidence on which to establish recommendations for the definition of bronchiolitis.

A rare attempt at using a structured approach to obtain a definition of disease was reported by a local clinical guideline development group in Nottingham, UK.[726] A Delphi panel of 50 physicians and nurses (mostly pediatricians) reached 90% consensus on bronchiolitis as 'a seasonal viral illness characterized by fever, nasal discharge and dry, wheezy cough', with 'fine inspiratory crackles and/or high pitched expiratory wheeze'. This definition could be considered in children below two years of age; no further details were given on any other demographic, clinical or biological parameters. Although a summary of evidence was given to the panel, this definition was clinically-oriented and it is not clear whether its implications for research were discussed. Unfortunately, no other such structured approaches are

Table 1.4: Differences in bronchiolitis definitions in landmark opinion papers, recent clinical practice guidelines and large clinical trials

Document			Bronchiolitis definition				
Type	**Authors**	**Date**	**Nr of episodes**	**Age**	**Auscultatory findings**	**Other defining symptoms and signs**	**Other features**
Opinion paper	Court	1973	no specific limit	no specific limit; refers infants, <6M	wheezing, rhonchi and rales	upper respiratory features; low fever; rapid respiration, dyspnoea; chest distension	increased pulmonary translucency on chest radiograph
	Mcintosh	1976	no specific limit	usually <12M	wheeze	rhinorrhea, cough, dyspnea	NR
	McConochie	1983	first	<24M	expiratory wheezing	acute onset, viral respiratory illness	NR
Guideline	Lakhanpaul et al (Nottingham) ; Scottish Intercollegiate Guidelines Network*	2006	no specific limit	no specific limit; refers mainly <24M	fine inspiratory crackles and/or high pitched expiratory wheeze	fever, nasal discharge and dry, wheezy cough	NR
	American Academy of Pediatrics	2006	no specific limit	no specific limit; refers mainly <24M	wheezing, crackles,	rhinitis, tachypnea, cough, use of accessory muscles, and/or nasal flaring	NR
	Green et al (South African guideline)	2010	no specific limit	no specific limit; refers mainly <24M	wheezing	feed poorly, mild upper respiratory tract infection, low-grade fever, hyperinflation of the chest	NR
Clinical trial	Corneli	2007	first	2-12M	wheezing	NR	NR
	Plint	2009	first	1.5-12M	wheezing	signs of an upper respiratory tract infection	NR
	Skjerven#	2013	first or second	<12M	wheezing, rhonchi and rales	upper respiratory features; low fever; rapid respiration, dyspnoea; chest distension	increased pulmonary translucency on chest radiograph

*the SIGN guideline directly references the Nottingham guideline consensus definition
#this clinical trial uses part of Court's clinical criteria

known, and clinicians and researchers have repeatedly faced the dilemma of how to operationalize disease definition in the absence of clear guidance. The impact is considerable; in clinics, this has led to the use of the same diagnostic label in young children with important differences in their demographic, history and physical examination features.[55] Variability between centers in diagnostic labeling of lower respiratory tract infections (e.g. as bronchiolitis, asthma or wheezing) has

been shown to influence treatment practices, and may explain an important part of persistent practice variation in management of bronchiolitis.[55,556] It may also affect the accuracy of diagnostic coding (e.g. ICD codes), which is used to define bronchiolitis as an outcome in many prognostic studies. For all these motives, it is not surprising that many controversies regarding the interpretation of evidence on management and treatment options are often attributed to the details of disease definition.[590,727,728]

In this regard, it is interesting to note how recent the history of bronchiolitis in formal medical coding and scientific terminology is. The term bronchiolitis only appeared in the 8th revision of the WHO-led ICD, published in 1965, included in code 466 "Acute bronchitis and bronchiolitis".[729] A separate diagnosis code (466.1) was included in the 9th revision (1975), with a later addition of a specific RSV code for the first ICD10 version in 1990, and the extended ICD9 - Clinical Modification by the US National Center for Health Statistics. Curiously, the uptake of specific codes for different viral agents has lagged, e.g. metapneumovirus was only the second agent to be included in the ICD classification, in 2010. Similarly, the US National Library of Medicine Medical Subject Heading term for bronchiolitis was introduced as "viral bronchiolitis" in 1967, under the heading "bronchitis", while "Respiratory Syncytial Viruses" was created in 1977 (information obtained from the National Library of Medicine database). In 1988 a subheading "bronchiolitis" was created, in order to accommodate both "bronchiolitis obliterans" and "viral bronchiolitis". This latter subdivision highlights the confusing overlap with the group of complex histopathologic bronchiolar disorders for which no single classification scheme has been widely accepted.[730]

Importantly, terminology and nosology issues have often been attributed to a worldwide geographical divide in clinical teaching and practice as to what exactly constitutes bronchiolitis.[2,4,721,722] On the one hand, a North American definition is said to favor a first episode, with wheeze as a clinical finding, in up to one- or two-year-olds. Conversely, hallmarks of bronchiolitis in the UK and Australia, include crackles/crepitations in younger infants, with or without wheeze. Other countries in Europe and worldwide would possibly follow one of these different perspectives. These different perspectives are partly reflected in opinion papers, medical textbook definitions and some management guidelines across countries. Overlapping labels

with conditions that may also be considered as distinct differential diagnoses is also problematic. The North American definition overlaps with labels such as 'virus-induced wheeze' and 'wheezy bronchitis' in the UK; conversely, the UK definition could be labelled 'pneumonia' in the US.[4,721,723] In both cases, children with predominant wheezing might be classified as having reactive airways disease or being early asthmatics. However, empirical evidence to support these differences in perspectives is scarce, and lack of agreement may also exist at a regional and individual level.

Disease terminology is further complicated by differences in the nomenclature, identification and interpretation of respiratory sounds. Although there have been historical efforts to standardize the terminology of adventitious sounds, variation persists.[377,378,731] Thus the terms 'crackles', 'rales' or 'crepitations' are used by different authors, but are not necessarily interchangeable; different qualities of a same sound, e.g. high- or low-pitched wheeze, may also be used. Further, reliability of stethoscope examination has been shown to be poor to moderate for adventitious sounds in young children with LRTIs such as bronchiolitis.[381] In parallel, audible respiratory sounds in children are particularly prone to confusion by parents but also by health professionals, as they may be perceived and named differently.[379] Fernandes and other authors have showed this is a relevant issue with wheezing, which is a key symptom and sign driving both diagnostic and therapeutic decisions in bronchiolitis.[731-733] This imprecision is a major problem, which may lead for example to misclassification of previous episodes of wheezing, one of the key parameters in defining bronchiolitis.

Ultimately, the critical questions when dealing with definition of disease include:

1. how consistent is it with different manifestations and at various levels of severity;
2. how well does it reflect underlying pathogenesis; and finally
3. how does it impact aspects of epidemiology, diagnosis, prognosis, and treatment.

Some evidence exists that variability in key clinical parameters of bronchiolitis definition may influence some of these aspects. For example, Elphick and colleagues categorized infants hospitalized with RSV infection as 'acute bronchiolitis' (based on findings of widespread crepitations) or 'wheeze-associated

viral illness' at admission, and at 3 years of age found a predictable increase in cough and wheeze with intercurrent viral infections but no increase in atopy and asthma in those admitted with acute bronchiolitis, as opposed to wheezers who had increased persistent symptoms, use of inhaled steroids and allergic sensitization.[734] This suggests that the acoustic characteristics of these two adventitious sounds and their pathological correlates might be markers of distinct host responses in bronchiolitis. Further, children hospitalized for wheezing at older ages, i.e. between 12 and 24 months of age, as well as those with recurrent episodes, have a higher risk for having asthma.[725] In turn, Jartti et al showed both age of the child and number of episodes are associated with viral etiology and atopic characteristics, both of which may also influence short- and long-term respiratory outcomes.[95]

This inevitably leads to the close links and unclear boundaries between bronchiolitis and wheezing disorders. As previously mentioned, bronchiolitis may be the first or one of many episodes of wheezing with heterogeneous biological, genetic, viral or environmental determinants. Some authors suggest that when defining bronchiolitis we should consider prognostic factors for recurrent wheezing and asthma, since these likely reflect different underlying disease entities with distinct immunopathogenesis (e.g. underlying inflammation, previous lung function), and possibly treatment response (e.g. efficacy of corticosteroid or bronchodilator).[95,457,725] For example, restricted definitions of bronchiolitis have been proposed focused on younger infants (e.g. below 6 or 12 months), first episodes, absence of allergic diseases, or isolation of specific viruses; this might allow studies to include more homogenous populations and less asthma-prone children with preexisting inflammation.[95,725] However, it must be emphasized that our current ability to predict which trajectory a child with bronchiolitis will follow is limited.[527,735] Further, what we call preschool recurrent wheezing disorders and asthma are also highly variable conditions in both their clinical presentation and time course.[462] It is recognized that disease labels in inflammatory airway disease are imprecise, and the accuracy of disease classification matters less.

To circumvent this obstacle, research on wheezing disorders has been progressively focusing on phenotype description as opposed to specific disease labels.[736] The interpretation of the term 'phenotype' is variable, and can include sets of observable or measurable traits, pragmatic constructs with prognostic or therapeutic

relevance, or a more fundamental meaning related to separate disease entities (Table 1.5).[462] Some distinguish phenotypes as 'clinically observable characteristics' of a disease without direct relationship to an underlying pathophysiology, from 'endotypes', subtypes of a disease defined by an intrinsically 'distinct pathogenetic mechanism'.[737,738] Airway disease is composed of many domains or dimensions, e.g. environmental triggers, clinical features, physiology, pathology, immunology, cell biology, genetic background and response to treatment, and each of these dimensions contains measurable variables. Phenotype definitions have used either a single or a few of these disease dimensions (one-dimensional), or a wide range of them (multi-dimensional).[462] "Hypothesis-free'' approaches are being increasingly used in all fields of airway disorders, by applying multivariate methods such as cluster and factor analyses to observed features from large scale cohort clinical studies, in order to identify these phenotypes that might better reflect underlying biological pathways.[462,739-741] Such methods allow phenotypes to be identified in a data-driven and might therefore minimize the subjectivity involved in selecting the features. However, clustering methods may distinguish groups regardless of whether they exist in the population as true entities or not. Validating such phenotypes requires evidence on their association with disease severity, prognosis and treatment response. Further, given the variability in concepts among clinicians and among researchers, plausible definitions and models of disease consisting of predefined disease entities are needed.[462] Spycher and colleagues have obtained such models by using a panel of clinicians familiar with pediatric wheezing disorders and basing them on their clinical experience.[742] Phenotype-based approaches to the definition and classification of bronchiolitis have been implicitly or explicitly suggested, but rarely explored. Everard has focused on auscultatory sounds (wheeze vs crackles) during bronchiolitis as a dimension that may suggest distinct entities.[4,167,734] We can envision that phenotypes of bronchiolitis could be evaluated based on different dimensions and traits of disease, e.g. demographics, clinical symptoms, physiological variables, markers of inflammation.

Table 1.5: Different usages of the term 'phenotype' (based on Spycher et al, with permissions)

Usage	Description	Example of usage in airways disorders	Possible use in bronchiolitis
Any observable trait (partial phenotype)	Includes signs, symptoms, measurements and biological markers	Wheeze and cough Increase specific IgE Bronchial hyperresponsiveness	Age Number of episodes Auscultatory findings Specific virus or co-infection
Clinically useful grouping	Defines groups that differ with respect to features of interest: e.g. risk factors, response to treatment, prognosis; may not correspond to underlying entity	Difficult or severe asthma	Based on one or more of the above dimensions/ traits, if validated
Hypothesized disease entity	Defines a condition that is thought to represent a distinct disease entity	Atopic asthma Virus-induced wheeze	Early asthma

In summary, there are many possible sources of heterogeneity in bronchiolitis definition with considerable impact in clinical practice and research, but scarce empirical evidence exploring them. Efforts to standardize definitions and subgrouping phenotypes of interest are needed; understanding physician perspectives would be a starting point before validating such classifications for epidemiology, disease severity, prognosis and treatment response purposes. In Chapter 4 of this thesis we will assess how pediatricians and general practitioners perceive the definition of bronchiolitis.

1.3 OBJECTIVES AND OUTLINE OF THE THESIS

The general aims of this thesis are to provide a comprehensive and integrated perspective of current evidence on bronchiolitis interventions, and to address two key shortcomings in clinical trial design and interpretation in this field, i.e. disease definition and outcome selection and measurement.

The following research questions regarding the management (1-3), outcome measurement (4-6), and definition (7,8) of acute bronchiolitis are addressed:

1. What is the efficacy and safety of bronchodilators (β2 agonists, adrenaline, anticholinergics) as compared to placebo?
2. What is the efficacy and safety of corticosteroids as compared to placebo?
3. How do these therapies compare between them, and is there a positive or negative effect of combining them?
4. Which outcome domains and measurement instruments have been used in previous clinical trials of bronchiolitis?
5. Which outcomes are considered most important to physicians when measuring bronchiolitis?
6. What are the measurement properties (i.e. validity, reliability and responsiveness) of two of the most commonly used instruments to assess bronchiolitis (RDAI and RACS scales)?
7. How do physicians define bronchiolitis, do definitions differ between specialty (pediatricians vs general practitioners), and how well do they match the ones frequently used in clinical trials?
8. Are there any meaningful dimensions underlying physician definitions of bronchiolitis?

Our first objective was to conduct a comparative effectiveness systematic review with network meta-analysis on the efficacy and safety of bronchodilators and corticosteroids for the treatment of acute bronchiolitis **(Chapter 2)**. In particular, we used a comprehensive approach to systematically evaluate and compare the evidence on use of β2 agonists, adrenaline, anticholinergics and corticosteroids,

alone or combined (with or without a fixed protocol), and we assessed the relative efficacy and safety of these interventions using network meta-analysis **(Chapter 2.1)**. Detailed analysis on corticosteroids is presented in a Cochrane review on corticosteroids, which was updated during the period of this thesis **(Chapter 2.2)**.

Based on the panorama of current evidence provided by this first project and a recent overview of reviews to which we contributed, our second objective was to identify and characterize outcome domains and measurement instruments reported in clinical trials **(Chapter 3.1)**. We identified the RDAI and RACS as the two most frequently used instruments in included trials, for both of which there is limited evidence on their suitability as outcome measures. **Chapter 3.2** reports the results of a study whose objective was to assess the measurement properties of the RDAI and the RACS. In particular, we evaluated and compared the validity, reliability and responsiveness of both scales using data from two large studies, one RCT and one prospective cohort.

As a first step to the development of a core outcome set, we assessed the perspectives of physicians on important outcomes for bronchiolitis trials **(Chapter 4)**. We present results from a nationwide electronic survey with pediatricians and general practitioners to characterize and compare their views on important outcomes in bronchiolitis.

Another objective of this survey was to evaluate the perspectives of physicians on the definition of bronchiolitis (also presented in **Chapter 4**). Further, we used exploratory factor analysis to examine whether any meaningful dimensions could be distinguished underlying these perspectives, in order to identify items and phenotypes that need to be addressed in a standardized definition of bronchiolitis.

Finally, a general discussion of all findings in the present thesis is presented in **Chapter 5**. Here, implications for clinical practice and directions for future research in each of the three topics addressed (therapeutic management, outcome measurement, and definition) are given.

CHAPTER 2

EVIDENCE ON CORTICOSTEROIDS AND BRONCHODILATORS FOR BRONCHIOLITIS

2.1 Corticosteroids And Bronchodilators For Acute Bronchiolitis In The First Two Years Of Life: Systematic Review And Meta-Analysis

2.2 Corticosteroids For Acute Viral Bronchiolitis In Infants And Young Children

2.1 CORTICOSTEROIDS AND BRONCHODILATORS FOR ACUTE BRONCHIOLITIS IN THE FIRST TWO YEARS OF LIFE: SYSTEMATIC REVIEW AND META-ANALYSIS

Adapted from:
Hartling L, Fernandes RM, Bialy L, Milne A, Johnson D, Plint A, Klassen TP, Vandermeer B. Steroids and bronchodilators for acute bronchiolitis in the first two years of life: systematic review and meta-analysis. British Medical Journal, 2011;342:d1714.

Here we present an adapted version of this publication. The following data is shown in the Appendix A1 of this thesis: list of included studies; complete search strategies; tables with GRADE assessments.

Authors

Lisa Hartling (1), Ricardo M Fernandes (2), Liza M Bialy (1), Andrea Milne (1), David W Johnson (3), Amy C Plint (4), Terry P Klassen (5), Ben Vandermeer (1)

Affiliations

1. Alberta Research Centre for Health Evidence, Department of Pediatrics, University of Alberta
2. Gulbenkian Program for Advanced Medical Education; Child and Family Department, Hospital de Santa Maria; Clinical Pharmacology and Therapeutics, Institute of Molecular Medicine, Lisbon, Portugal
3. Department of Pediatrics and Department of Physiology and Pharmacology, University of Calgary and Alberta Children's Hospital, Calgary
4. Department of Pediatrics and Department of Emergency Medicine, University of Ottawa, Ottawa
5. Manitoba Institute of Child Health, Department of Pediatrics and Child Health, University of Manitoba, Winnipeg

ABSTRACT

Objective

To evaluate and compare the efficacy and safety of bronchodilators and steroids, alone or combined, for the acute management of bronchiolitis in children aged less than 2 years.

Design

Systematic review and meta-analysis. Data sources Medline, Embase, Central, Scopus, PubMed, LILACS, IranMedEx, conference proceedings, and trial registers.

Inclusion criteria

Randomized controlled trials of children aged 24 months or less with a first episode of bronchiolitis with wheezing comparing any bronchodilator or steroid, alone or combined, with placebo or another intervention (other bronchodilator, other steroid, standard care).

Review methods

Two reviewers assessed studies for inclusion and risk of bias and extracted data. Primary outcomes were selected by clinicians a priori based on clinical relevance: rate of admission for outpatients (day 1 and up to day 7) and length of stay for inpatients. Direct meta-analyses were carried out using random effects models. A mixed treatment comparison using a Bayesian network model was used to compare all interventions simultaneously.

Results

48 trials (4897 patients, 13 comparisons) were included. Risk of bias was low in 17% (n=8), unclear in 52% (n=25), and high in 31% (n=15). Only adrenaline (epinephrine) reduced admissions on day 1 (compared with placebo: pooled risk ratio 0.67, 95% confidence interval 0.50 to 0.89; number needed to treat 15, 95% confidence interval 10 to 45 for a baseline risk of 20%; 920 patients). Unadjusted results from a single large trial with low risk of bias showed that combined dexamethasone and adrenaline reduced admissions on day 7 (risk ratio 0.65, 0.44 to 0.95; number needed to treat 11, 7 to 76 for a baseline risk of 26%; 400

patients). A mixed treatment comparison supported adrenaline alone or combined with steroids as the preferred treatments for outpatients (probability of being the best treatment based on admissions at day 1 were 45% and 39%, respectively). The incidence of reported harms did not differ. None of the interventions examined showed clear efficacy for length of stay among inpatients.

Conclusions

Evidence shows the effectiveness and superiority of adrenaline for outcomes of most clinical relevance among outpatients with acute bronchiolitis, and evidence from a single precise trial for combined adrenaline and dexamethasone.

INTRODUCTION

Bronchiolitis is the most common disease of the lower respiratory tract during the first year of life.[1] Respiratory syncytial virus is the underlying cause of most bronchiolitis and this infection is associated with substantial morbidity in young children.[38,54] Ongoing research in bronchiolitis reflects both the burden of disease in developed and developing countries and a lack of clear evidence for its therapeutic management.[2] Previous studies have shown substantial variation in the management of acute bronchiolitis throughout the world, including the use of different bronchodilators (β2 agonists, adrenaline (epinephrine), anticholinergics) and steroids.[8,9,551,557] Some of this variation may be attributable to varying severity of disease or to different care settings and geographical location.

Several systematic reviews have assessed various treatments, including β2 agonists and anticholinergics, adrenaline, corticosteroids, hypertonic saline, antibiotics, surfactant, ribavirin, and chest physiotherapy.[10] These reviews have failed to provide convincing evidence to support any of these treatments in the acute management of bronchiolitis, and their routine use is not recommended by current clinical practice guidelines.[393,394,743] Despite implementation of these guidelines, bronchodilators especially are still frequently used.[744,745]

A 2003 report recommended rigorously designed, adequately sized randomized controlled trials on treatments that showed some potential for being efficacious, including nebulized bronchodilators (adrenaline, salbutamol, or ipratropium

bromide, alone or combined), oral or parenteral corticosteroids (preferentially dexamethasone), and inhaled corticosteroids (especially budesonide).[695] Two large trials examining some of these interventions have recently been completed. The largest trial ever published in this area, concerning 800 children in Canada, used a factorial design to examine adrenaline and dexamethasone, alone or combined, compared with placebo.[46] Another trial completed concurrently in the United States compared dexamethasone with placebo in a sample of 600 children.[45] These two large trials add substantially to the evidence and provide a strong signal for further synthesis work.[746]

These recent trials also raise new questions and potentially novel approaches to the acute management of bronchiolitis that warrant closer investigation. Specifically, one trial showed a 35% relative reduction on rates of admission to hospital with combined adrenaline and dexamethasone treatment compared with placebo.[46] Previously, a smaller trial was the first to show the effectiveness of oral dexamethasone in reducing hospital admissions in outpatients with acute bronchiolitis.[747] The unique feature of this trial, among others that did not show effectiveness, was that high dose steroids were administered along with a bronchodilator (salbutamol) according to a defined protocol rather than at the discretion of the attending physician. Although the interactive effect of steroids and bronchodilators has emerged as a potential treatment option, it has not been examined at the level of systematic review and placed in the context of other evidence.

Driven by recent evidence and current uncertainties in practice, we systematically evaluated and compared the efficacy and safety of bronchodilators (β2 agonists, adrenaline, anticholinergics) and steroids, alone or combined, for the acute management of bronchiolitis. We also determined the effectiveness of steroids with a fixed protocol for bronchodilator use compared with those in which the bronchodilator was given at the discretion of the attending physician, and bronchodilators given with and without steroids. By carrying out mixed treatment comparisons, we sought to improve on previous systematic reviews that focused primarily on pairwise, direct comparisons, often with the comparator being a placebo. Mixed treatment analysis is a relatively new development in the area of

evidence synthesis, with the advantage of combining data on different interventions for the same condition.[661,748]

METHODS

Through all stages of this work, we followed a protocol that was developed by the coauthors before the review began, in which all outcomes and analyses were specified a priori.

Search strategy

A medical research librarian searched Medline Ovid version (1950 to November week 2, 2009), Embase Ovid version (1980 to 2009 week 47), EBM Reviews — Cochrane Central Register of Controlled Trials (4th quarter 2009), LILACS—Latin American and Caribbean Center on Health Sciences Information (25 November 2009), PubMed (9 March 2009), Scopus (1823 to 25 November 2009), and IranMedEx (26 November 2009). We applied no restrictions on year or language. To identify unpublished studies and studies in progress, we searched conference proceedings for six relevant organizations and six clinical trials registers. Finally, we checked reference lists of relevant studies and previous reviews and contacted experts in the specialty.

Study selection

Studies were included if they were randomized controlled trials, involved inpatients or outpatients aged 24 months or less with bronchiolitis, and compared a bronchodilator (salbutamol or terbutaline, adrenaline, ipratropium bromide) or steroid (inhaled or systemic), or both combined, with another intervention (either placebo or another intervention including another bronchodilator or steroid). Bronchiolitis was defined as a physician diagnosed first episode of acute wheezing with respiratory distress and associated with clinical evidence of viral infection. We excluded studies in which any participants had a history of wheezing, respiratory distress, or a formal diagnosis of asthma. We chose to focus on first episodes of wheezing to deal with the possible overlap between bronchiolitis, recurrent wheezing, and asthma. We also excluded studies in the intensive care setting or with intubated or ventilated participants, and studies assessing longer courses of steroids started during the acute phase of bronchiolitis for the prevention of post-bronchiolitic wheezing. The primary outcomes, selected by the clinician authors a

priori based on clinical relevance, were rate of admissions at day 1 and day 7 for outpatient studies and length of stay in hospital for inpatient studies. Secondary outcomes included change in clinical score, oxygen saturation, respiratory rate, and heart rate; readmissions (for inpatients); return visits to the emergency department or any healthcare provider; and harms or adverse events.

Two reviewers independently screened the titles and abstracts to determine if an article met the inclusion criteria. The full text of studies classified as "relevant" or "unclear" were assessed independently by two reviewers using a standard form. Disagreements were resolved by consensus or adjudication by a third party.

Risk of bias assessment

Included studies were assessed using the Cochrane Collaboration risk of bias tool.[749] The tool consists of six domains (sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, and "other sources of bias"). Blinding and incomplete outcome data were assessed separately for the following types of outcomes: administrative, clinical or respiratory scores and other clinical variables, and others (for example, adverse events). Two reviewers independently assessed risk of bias of included studies. One reviewer assessed reports written in Turkish. Discrepancies were resolved by consensus among three reviewers.

Data extraction

Data were extracted using a standardized form (available from authors) and entered into Microsoft Excel (Microsoft, Redmond, WA). One reviewer extracted data and a second reviewer checked these for accuracy and completeness. Extracted data included study characteristics, inclusion and exclusion criteria, characteristics of participants, interventions (including the use of a fixed protocol for co-interventions), outcomes, and results. Reviewers resolved discrepancies by consensus or in consultation with a third party. All quantitative data were checked by the statistician during analysis.

Grading the body of evidence

Two reviewers independently graded the quality of the body of evidence for the comparisons deemed most clinically relevant. Assessments, based on a modified

GRADE approach, were completed for length of stay and admissions, change in clinical score, and adverse events.[659,750] Domains examined were risk of bias, consistency, directness, and precision. Decision rules were developed a priori based on clinical and methodological relevance and are available on request. Discrepancies were resolved through discussion. The overall strength of evidence was graded as high, moderate, low, or insufficient.

Statistical analysis

We considered studies of inpatients and outpatients separately, except for harms related data. Weighted mean differences were used to pool continuous variables when the same measurement scale was used (for example, heart rate) and standardized mean differences when different scales were used (for example, clinical scores). For pairwise meta-analysis, we used risk ratios to pool dichotomous variables. Data were combined using the DerSimonian-Laird random effects models in Review Manager version 5.0 (Cochrane Collaboration, Copenhagen, Denmark).[751] Results are reported with 95% confidence intervals, and statistical significance was set at $P<0.05$. Statistical heterogeneity was quantified using the I^2 statistic. A value greater than 50% was considered to be substantial heterogeneity.[752,753] We calculated numbers needed to treat using the final risk ratios and the simple average baseline risk across all included trials—that is, the number of events divided by the number of participants across the placebo arms of relevant studies. We also computed numbers needed to treat using the minimum and maximum baseline risk for the trials included in the meta-analysis. In our main analysis, for studies where groups received combined interventions that followed a protocol, we considered the common intervention across groups to "cancel out." For example, to obtain an overall main effect we considered a study comparing combined steroid and bronchodilator with bronchodilator alone in the comparison of steroid with placebo. Furthermore, two groups from factorial trials could contribute to the same analysis—for example, combined steroid and placebo compared with combined placebo and placebo, and combined steroid and bronchodilator compared with combined bronchodilator and placebo would both contribute to the comparison of steroid with placebo. The robustness of this assumption was tested by carrying out subgroup analyses comparing results in trials with co-interventions following a protocol versus trials with co-interventions at the discretion of the physician to

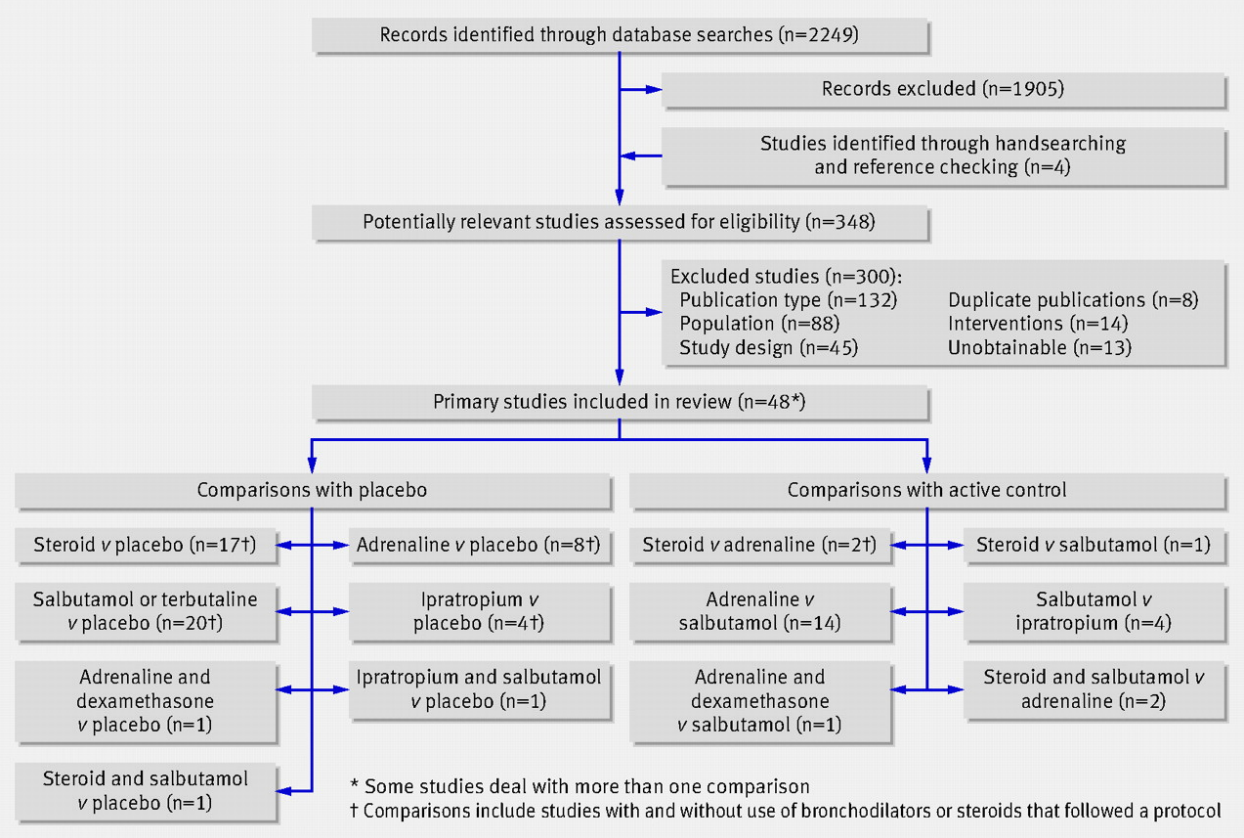


Figure 2.1. Flow diagram for study selection

explore potential additive (synergistic) or subtractive (antagonistic) effects. A priori we planned to do sensitivity analyses based on risk of bias (low vs unclear or high).

For the primary outcomes we carried out a mixed treatment analysis using a Bayesian network model to compare all interventions simultaneously and to use all available information on treatment effects in a single analysis.[748,754,755] Mean differences or log odds ratios were modeled using non-informative prior distributions. A normal prior distribution with mean 0 and large variance (10 000) was used for each of the trial means or log odds ratios, whereas their between study variance had a uniform prior with range 0 to 2 (admissions) or 0 to 10 (length of stay). These priors were checked for influence with sensitivity analyses. We carried out Markov Chain Monte Carlo simulations using WinBugs software to obtain simultaneous estimates of all interventions compared with placebo as well as estimates of which interventions were the best.[756] A burn-in sample of 20 000

iterations was followed by 200 000 iterations used to compute estimates. Results are reported with 95% credibility intervals. We considered all trial groups separately in the analysis. For example, a trial comparing steroid with placebo using a fixed protocol for bronchodilator use in both arms would contribute two arms to the mixed treatment analysis: combined steroid and bronchodilator and bronchodilator. Factorial trials contributed all four groups, and correlation between groups in such trials was factored into the computations. We checked the analyses for consistency using cross validation of all contrasts that had direct evidence.[757]

RESULTS

Figure 2.1 shows the flow of studies through the selection process. Forty eight studies totaling 4897 patients were included. Table 2.1 shows the comparisons made, the number of studies for each comparison by inpatient and outpatient population, the number of studies that provided data for our primary outcomes, the years of publication, and country of study. The drugs were administered in a variety of ways and varied across studies and interventions: corticosteroids –systemic (oral, intravenous, or intramuscular) or nebulized; adrenaline –nebulized; and bronchodilators –mostly nebulized. The risk of bias was low for eight studies (17%), unclear for 25 (52%), and high for 15 (31%). Twenty four studies only included infants aged less than 1 year.

Table 2.1: Overview of studies included in systematic review

Comparison by population	No of studies (patients)	No of studies with data on primary outcome	Years of publication (median)	Countries of study	Risk of bias
Steroid vs placebo:					
Inpatients	9 (772)	8	1996-2007 (2000)	UK (2), Israel, Belgium, Mexico, Canada, Thailand, Brazil, USA	1 low, 4 unclear, 4 high
Outpatients	8 (1778)	8	1998-2009 (2002/2004)	USA (2), Canada (2), Turkey (2), Israel, Paraguay	1 low, 4 unclear, 3 high
Adrenaline vs placebo:					
Inpatients	3 (330)	2	2002-3 (2002)	England, Canada, Australia	1 low, 1 unclear, 1 high

Table 2.1: Overview of studies included in systematic review					
Comparison by population	**No of studies (patients)**	**No of studies with data on primary outcome**	**Years of publication (median)**	**Countries of study**	**Risk of bias**
Outpatients	5 (526)	4	1995-2009 (2005)	Turkey (2), Iran, Canada, USA	2 low, 2 unclear, 1 high
Salbutamol* or terbutaline vs placebo:					
Inpatients	9 (488)	6	1991-2009 (1997)	Turkey (2), France, Saudi Arabia, Singapore, Canada, USA, Tunisia, Australia	1 low, 5 unclear, 3 high
Outpatients	11 (926)	6	1990-2008 (1998)	Canada (3), Turkey (3), USA (2), Egypt, India, Iran	2 low, 6 unclear, 3 high
Ipratropium vs placebo:					
Inpatients	3 (194)	2	1995-2008 (1997)	Saudi Arabia, Singapore, Turkey	3 unclear
Outpatients	1 (72)	1	1992	Canada	1 unclear
Adrenaline and dexamethasone vs other:					
Inpatients	0	NA	NA	NA	NA
Outpatients	2 (436)	2	2004, 2009	Turkey, Canada	1 low, 1 high
Ipratropium and salbutamol* vs placebo:					
Inpatients	0	NA	NA	NA	NA
Outpatients	1 (72)	1	1992	Canada	1 unclear
Steroid vs adrenaline:					
Inpatients	0	NA	NA	NA	NA
Outpatients	2 (444)	2	1995, 2009	Turkey, Canada	1 low, 1 high
Adrenaline vs salbutamol*:					
Inpatients	6 (433)	4	1993-2007 (2001/2002)	Canada (2), Jordan, Chile, Iran, India	1 low, 3 unclear, 2 high
Outpatients	8 (378)	6	1995-2007 (2004)	Turkey (3), USA (2), Israel, Iran, Canada,	4 low, 3 unclear, 1 high
Steroid vs salbutamol*:					
Inpatients	0	NA	NA	NA	NA
Outpatients	1 (45)	1	1995	Turkey	1 high
Salbutamol vs ipratropium:					
Inpatients	4 (192)	3	1995-2008 (2000)	Saudi Arabia, Singapore, Turkey (2)	3 unclear, 1 high
Outpatients	0	NA	NA	NA	NA
Steroid and salbutamol* vs other:					

Table 2.1: Overview of studies included in systematic review					
Comparison by population	**No of studies (patients)**	**No of studies with data on primary outcome**	**Years of publication (median)**	**Countries of study**	**Risk of bias**
Inpatients	0	NA	NA	NA	NA
Outpatients	2 (103)	2	1998, 2004	Turkey (2)	2 high

NA=Not applicable.

*Salbutamol has been used throughout to also refer to albuterol.

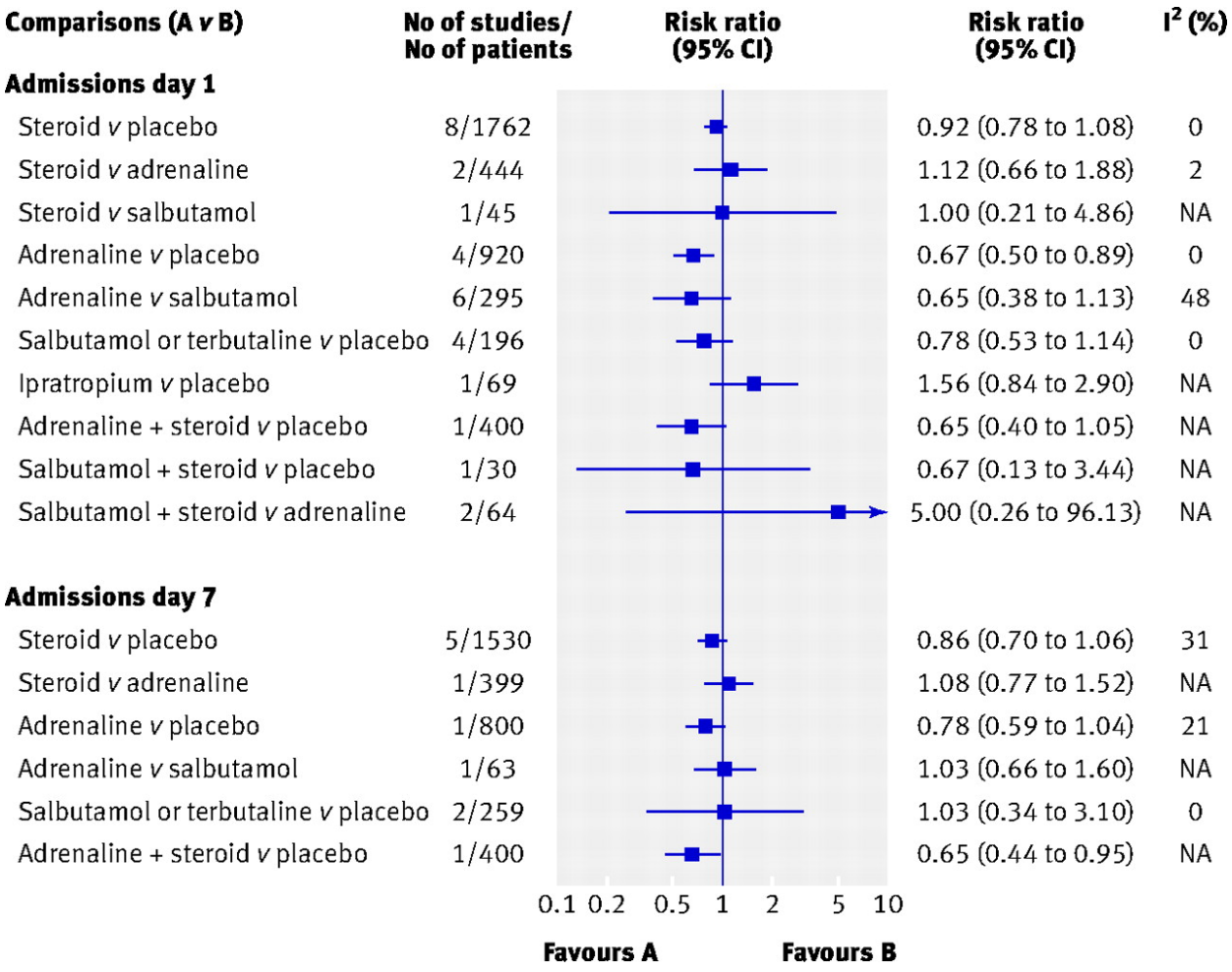


Figure 2.2. Results from meta-analysis of direct comparisons for admission rates from emergency department (day 1 and day 7) in outpatients. Only comparisons with quantitative results are shown

Outpatients

Figure 2.2 displays the effect estimates for the primary outcome of admission rates from the emergency department (day 1) for the different direct comparisons. The results were statistically significant for only one comparison, showing a reduction of 33% for adrenaline compared with placebo (pooled risk ratio 0.67, 95% confidence interval 0.50 to 0.89). The strength of evidence for this finding was considered moderate owing to lack of precision. The number needed to treat based on the average baseline risk of admission from all studies (20%) was 15 (95% confidence interval 10 to 45). The number needed to treat ranged from 4 (95% confidence interval 3 to 12, baseline risk 75%) to 20 (13 to 59, baseline risk 15%). The results were sensitive to risk of bias: when studies with an unclear risk of bias were removed, the pooled estimate for the two studies (n=842) at low risk of bias was no longer statistically significant (pooled risk ratio 0.77, 0.56 to 1.07). Subgroup analyses showed non-statistically significant differences between studies combining adrenaline with steroids that followed a protocol (pooled risk ratio 0.74, 0.45 to 1.23; one study, n=400) compared with those that did not follow a protocol (0.62, 0.40 to 0.94; four studies, n=520; ratio of risk ratios 1.19, 0.61 to 2.33). An effect of a similar magnitude was shown with combined adrenaline and dexamethasone compared with placebo (35%), but this did not reach significance (P=0.07) (pooled risk ratio 0.65, 0.4 to 1.05; one study, n=400).

Figure 2.3 illustrates the comparisons and number of studies for each that were examined in the mixed treatment comparison for admissions at day 1. Cross validation showed that the results from the mixed treatment analysis were consistent with direct evidence not differing significantly from indirect evidence for any of the paired comparisons where direct evidence was available. The mixed treatment comparison identified adrenaline alone and combined adrenaline and dexamethasone as the interventions with the highest probability of being most effective, with about half the odds of being admitted from the emergency department compared with placebo (Figure 2.4). The odds ratios were 0.48 (95% credibility interval 0.18 to 1.01) for adrenaline alone and 0.52 (0.15 to 1.57) for combined adrenaline and dexamethasone. Although this provides evidence on the relative efficacy of the different interventions, none of the interventions compared with placebo was statistically significant in this analysis.

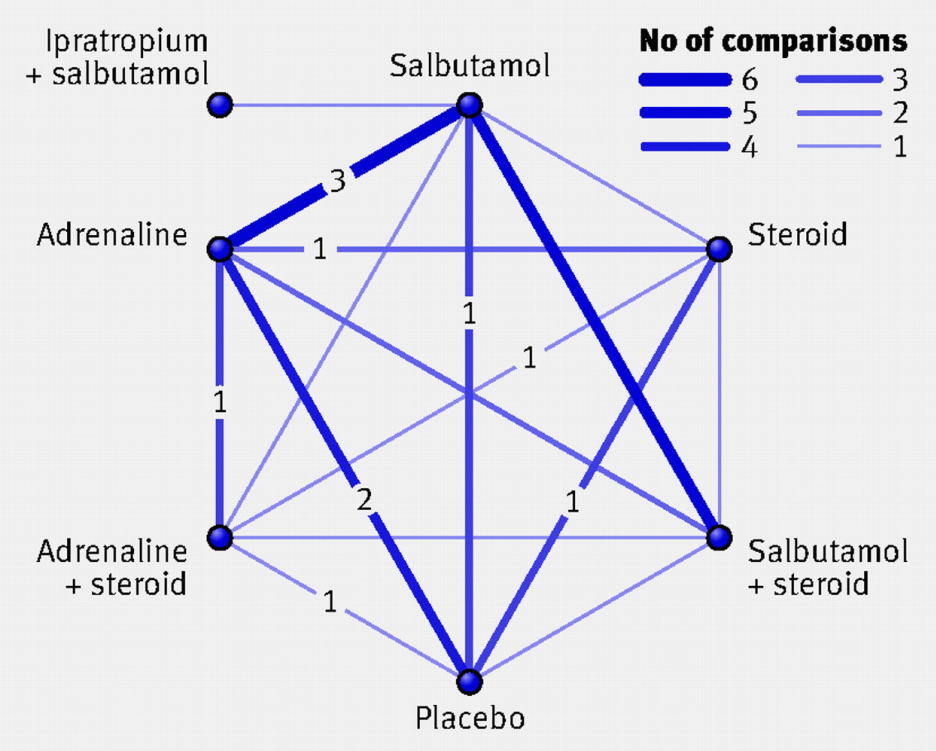


Figure 2.3. Comparisons (14 studies) contributing to mixed treatment analysis for admissions at day 1. Numerals within figure are studies at low risk of bias (four in total).

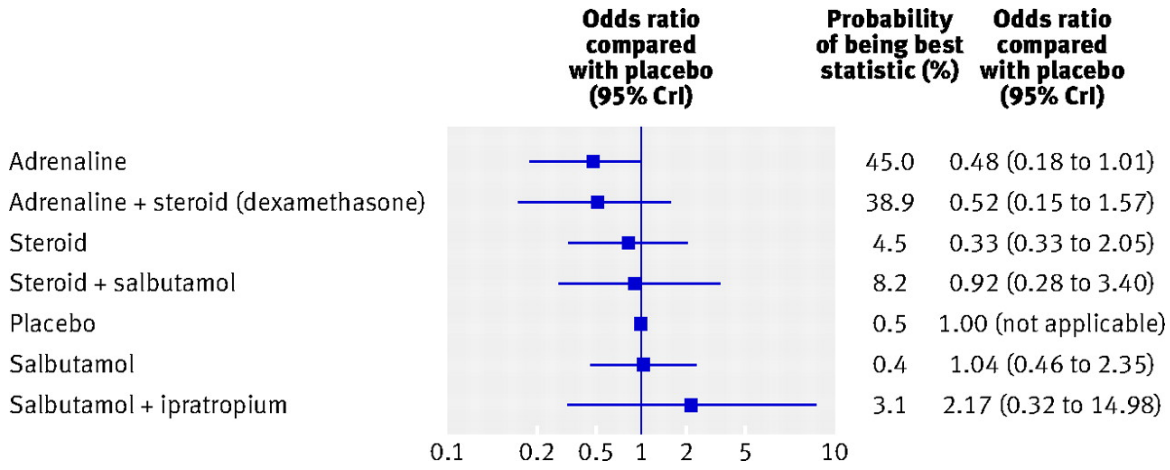


Figure 2.4. Results of mixed treatment analysis for admissions at day 1, showing probability ranking and probability of being best statistic.

Admission rates were also examined up to seven days after the emergency department visit using direct comparisons (Figure 2.2). One large study with low

risk of bias showed a statistically significant result for combined adrenaline and dexamethasone, with a 35% reduction compared with placebo (pooled risk ratio 0.65, 95% confidence interval 0.44 to 0.95); number needed to treat 11 (95% confidence interval 7 to 76). This result was based on a single large trial at low risk of bias; however, the study was factorial and the authors did not anticipate or hypothesize an effect for the combined adrenaline and dexamethasone group a priori.16 The strength of evidence for this finding, based on the modified GRADE system, is considered low, as evidence came from only one study with relatively few events. The overall results for steroids compared with placebo and for adrenaline compared with placebo were not statistically significant; however, subgroup analyses examining use of bronchodilators or steroids that followed a protocol showed some important effects (data not shown). Specifically, adrenaline along with steroids that followed a protocol compared with placebo and steroids showed a statistically significant reduction of 33% (pooled risk ratio 0.67, 95% confidence interval 0.45 to 0.98). Also, steroids with use of bronchodilators (adrenaline or salbutamol) that followed a protocol compared with placebo and bronchodilators showed a similar magnitude of effect (32%) but did not reach statistical significance (pooled risk ratio 0.68, 95% confidence interval 0.44 to 1.05; P=0.08).

Mixed treatment comparison for admissions up to day 7 identified steroids with bronchodilators (adrenaline or salbutamol) as the interventions with the highest probability of being most effective, although the credibility intervals were wide and do not rule out the possibility of no effect (Figure 2.3 extra).

Table 2.2 presents the results from pairwise meta-analysis for change in clinical score. Only nine of the 25 comparisons were statistically significant, and in six of these adrenaline or adrenaline and dexamethasone was the preferred treatment. Compared with placebo, significant benefits were observed for adrenaline at 60 and 120 minutes, combined adrenaline and dexamethasone at 60 minutes, and salbutamol at 60 minutes. Adrenaline showed significant benefits compared with steroids at 60 minutes and salbutamol at 3-10 days. Combined adrenaline and dexamethasone was also superior to salbutamol at 3-10 days. The other two significant results were from one small study at unclear risk of bias showing benefits of salbutamol compared with steroids. The results for other clinical variables were

consistent with the findings of admission rates and clinical score or provided little additional information (data available from authors). The incidence of return visits did not differ for any of the five comparisons where data were available (steroid vs placebo, steroid vs adrenaline, adrenaline vs placebo, adrenaline vs salbutamol, combined adrenaline and dexamethasone vs placebo), although there was only one or two studies within each comparison for this outcome.

Table 2.2: Results of direct comparisons for change in clinical score among outpatients

Comparison	Time point	No of studies (patients)	Standardised mean difference (95% CI)	I^2
Steroid vs placebo	1 hour	4 (1006)	−0.04 (−0.16 to 0.09)	0
	2 hour	3 (214)	−0.17 (−0.55 to 0.21)	43
	3-6 hours	4 (808)	−0.14 (−0.50 to 0.21)	68
	12-24 hours	1 (69)	0.13 (−0.51 to 0.76)	36
	3-10 days	4 (224)	−0.20 (−0.61 to 0.21)	55
Steroid vs adrenaline	1 hour	2 (442)	0.31 (0.12 to 0.50)*	0
	2 hours	1 (45)	0.35 (−0.27 to 0.98)	NA
	3-6 hours	1 (45)	0.42 (−0.20 to 1.05)	NA
Steroid vs salbutamol	1 hour	1 (45)	0.65 (0.01 to 1.28)†	NA
	2 hours	1 (45)	0.36 (−0.27 to 0.98)	NA
	3-6 hours	1 (45)	0.70 (0.06 to 1.34)†	NA
Adrenaline vs placebo	1 hour	4 (900)	−0.45 (−0.66 to −0.23)*	40
	2 hours	1 (30)	−0.83 (−1.58 to −0.08)*	NA
Adrenaline vs salbutamol	1 hour	6 (248)	−0.11 (−0.36 to 0.14)	0
	2 hours	4 (207)	−0.09 (−0.37 to 0.18)	0
	12-24 hours	1 (69)	−0.21 (−0.86 to 0.44)	41
	3-10 days	1 (69)	−0.50 (−0.98 to −0.02)*	0
Salbutamol vs placebo	1 hour	8 (565)	−0.49 (−0.96 to −0.01)†	86
	2 hours	2 (100)	−0.04 (−1.07 to 0.99)	84
	3-6 hours	1 (60)	−0.79 (−2.53 to 0.95)	90
Ipratropium vs placebo	2 hours	1 (69)	−0.14 (−0.61 to 0.33)	NA
Adrenaline and dexamethasone vs placebo	1 hour	1 (399)	−0.34 (−0.54 to −0.14)‡	NA

Table 2.2: Results of direct comparisons for change in clinical score among outpatients

Comparison	Time point	No of studies (patients)	Standardised mean difference (95% CI)	I^2
Adrenaline and dexamethasone vs salbutamol	2 hours	1 (35)	−0.17 (−0.87 to 0.52)	NA
	12-24 hours	1 (35)	0.00 (−0.70 to 0.70)	NA
	3-10 days	1 (35)	−1.22 (−1.98 to −0.46)‡	NA
Steroid and salbutamol vs placebo	1 hour	1 (30)	−0.34 (−1.75 to 1.07)	NA
	2 hours	1 (30)	−0.67 (−2.04 to 0.70)	NA
	3-6 hours	1 (30)	−1.08 (−2.43 to 0.27)	NA
Steroid and salbutamol vs adrenaline	1 hour	1 (30)	0.36 (−0.36 to 1.08)	NA
	2 hours	2 (64)	0.25 (−0.26 to 0.77)	0
	12-24 hours	1 (34)	0.30 (−0.43 to 1.02)	NA
	3-10 days	1 (34)	−0.16 (−0.88 to 0.56)	NA

NA=not applicable.

*Results favour adrenaline.

†Results favour salbutamol.

‡Results favour combined adrenaline and dexamethasone.

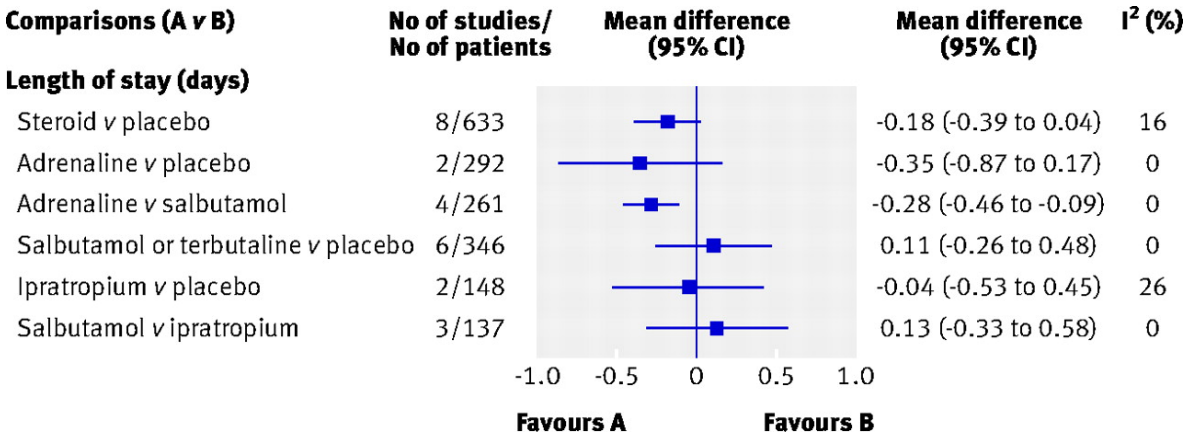


Figure 2.5. Results from meta-analysis of direct comparisons for length of stay in inpatients. Only comparisons with quantitative results are shown.

Inpatients

Figure 2.5 displays the effect estimates for the primary outcome of length of stay for the different direct comparisons. Only one comparison was statistically significant,

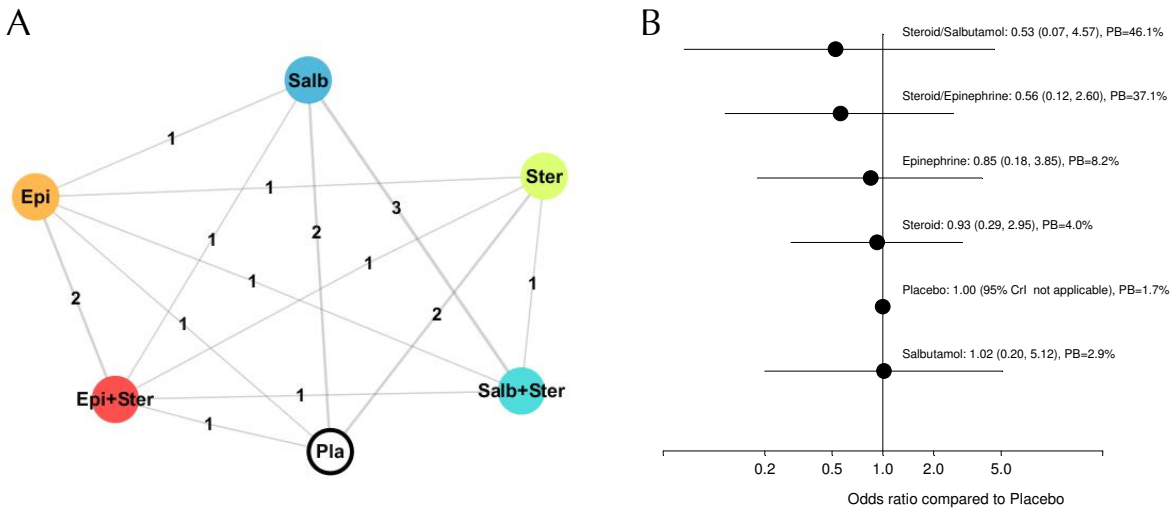


Figure 2.3 (extra). Comparisons contributing to mixed treatment analysis for admissions up to day 7 among outpatients - network geometry (A) and forrest plot with odds ratio compared with placebo and 95% credibility intervals, probability ranking and probability of being best statistic (B) [In Panel A, each treatment is shown by a node, and comparisons between treatments are shown with links between the nodes; the width of the lines reflect the number of comparisons which is also shown. Salb=salbutamol; Epi=epinephrine; Ster=steroid; Pla=placebo]; in panel B, PB=probability of being best]

showing a shorter length of stay for adrenaline compared with salbutamol (mean difference −0.28 days, 95% confidence interval −0.46 to −0.09). The strength of evidence for this comparison is considered moderate. However, the practical implications of this result need to be considered alongside the finding that adrenaline showed no significant benefit compared with placebo. Furthermore, this finding was sensitive to risk of bias: only one study for this comparison was at low risk of bias and the result was not significant (mean difference −0.07 days, 95% confidence interval −1.01 to 0.88). The results showed high strength of evidence of no difference for steroid compared with placebo overall. Subgroup analyses showed a significant difference for steroids with use of bronchodilators that followed a protocol (mean difference −0.12 days, −0.23 to −0.00); however, the magnitude of effect is not considered clinically important.

Mixed treatment comparison identified combined adrenaline and dexamethasone as the preferred treatment (Figures 2.6 and 2.7). This finding was driven by one small study at high risk of bias. The confidence interval was wide and did not rule out the potential for no effect. Moreover, the mixed treatment comparison shows

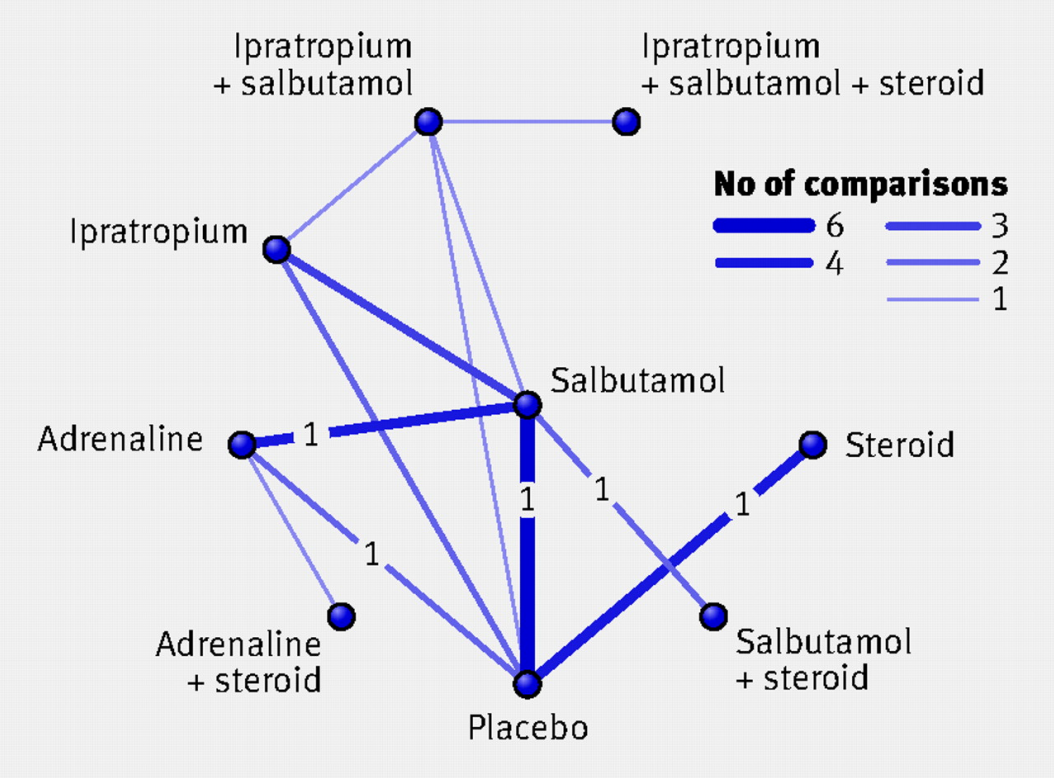


Figure 2.6. Comparisons (19 studies) contributing to mixed treatment analysis for length of stay. Numerals within figure are studies at low risk of bias (two in total).

that none of the interventions examined show clear efficacy in terms of length of stay among the inpatient population.

In terms of change in clinical score (Table 2.3), few differed statistically significantly (5/23 comparisons). Significant benefits were observed for adrenaline compared with salbutamol at 60 and 120 minutes, steroids compared with placebo at 3-6 and 6-12 hours, and salbutamol or terbutaline compared with placebo at 6-12 hours. The strength of evidence for these findings is limited by risk of bias, inconsistency (or unknown consistency owing to limited numbers of studies within individual comparisons), and lack of precision. The results for other clinical symptoms provided little additional or inconsistent information (data available from authors). Data for return visits and readmissions were available for four comparisons (steroid vs placebo, adrenaline vs placebo, adrenaline vs salbutamol, and salbutamol vs placebo). No significant differences were found although only one or two studies were available for each comparison.

Harms

Sixteen studies provided data on short term adverse effects. No studies examined, or were necessarily designed to examine, long term adverse effects, such as cognitive injury. The types of adverse effects that were most commonly searched for (or reported on) included pallor, vomiting, tremors, hypertension, tachycardia, and infections. In general, the incidence of adverse effects was low and no important differences were observed between groups across the studies.

Table 2.3: Results of direct comparisons for change in clinical score among inpatients by comparison

Comparison	Time point	No of studies (No of patients)	Standardised mean difference (95% CI)	I^2
Steroid vs placebo	3-6 hours	1 (174)	−1.03 (−1.87 to −0.19)*	NA
	6-12 hours	3 (269)	−0.62 (−1.00 to −0.23)*	10
	12-24 hours	3 (264)	−0.28 (−0.66 to 0.09)	41
	1-3 days	4 (271)	−0.53 (−1.14 to 0.08)	70
Adrenaline vs placebo	1 hour	2 (232)	−0.04 (−0.49 to 0.40)	46
Adrenaline vs salbutamol	1 hour	4 (248)	−0.79 (−1.45 to −0.13)†	79
	2 hours	1 (140)	−0.52 (−0.86 to −0.18)†	NA
Salbutamol or terbutaline vs placebo	1 hour	5 (223)	−0.20 (−0.76 to 0.35)	76
	2 hours	2 (68)	−0.78 (−2.53 to 0.98)	91
	3-6 hours	1 (89)	−0.20 (−0.61 to 0.22)	0
	6-12 hours	2 (136)	−0.81 (−1.21 to −0.40)‡	25
	12-24 hours	2 (136)	−0.21 (−0.62 to 0.20)	31
	1-3 days	3 (195)	−0.06 (−0.47 to 0.36)	53
Ipratropium vs placebo	1 hour	1 (89)	−0.11 (−0.53 to 0.31)	0
	3-6 hours	1 (89)	0.06 (−0.39 to 0.51)	13
	6-12 hours	2 (134)	−0.21 (−0.80 to 0.37)	65
	12-24 hours	3 (193)	−0.27 (−0.61 to 0.06)	27
	1-3 days	3 (193)	0.05 (−0.38 to 0.49)	56
Salbutamol vs ipratropium	1 hour	1 (43)	−0.22 (−0.82 to 0.38)	NA
	3-6 hours	1 (43)	0.20 (−0.40 to 0.80)	NA
	6-12 hours	3 (123)	0.16 (−0.40 to 0.72)	59

Table 2.3: Results of direct comparisons for change in clinical score among inpatients by comparison

Comparison	Time point	No of studies (No of patients)	Standardised mean difference (95% CI)	I^2
	12-24 hours	4 (183)	−0.24 (−0.54 to 0.06)	3
	1-3 days	4 (183)	−0.10 (−0.39 to 0.19)	0

NA=not applicable.
*Results favour steroid.
†Results favour adrenaline.
‡Results favour salbutamol or terbutaline.

DISCUSSION

Previous syntheses provide little conclusive evidence to support the choice of different treatment options in the acute management of bronchiolitis. By examining steroids and bronchodilators in a single systematic review and supplementing the standard meta-analysis with mixed treatment comparisons, this review pro- vides some important directions for clinical practice and future research. Adrenaline seems to be beneficial for short term outcomes among outpatients, including admission rates from the emergency department. Furthermore, adrenaline

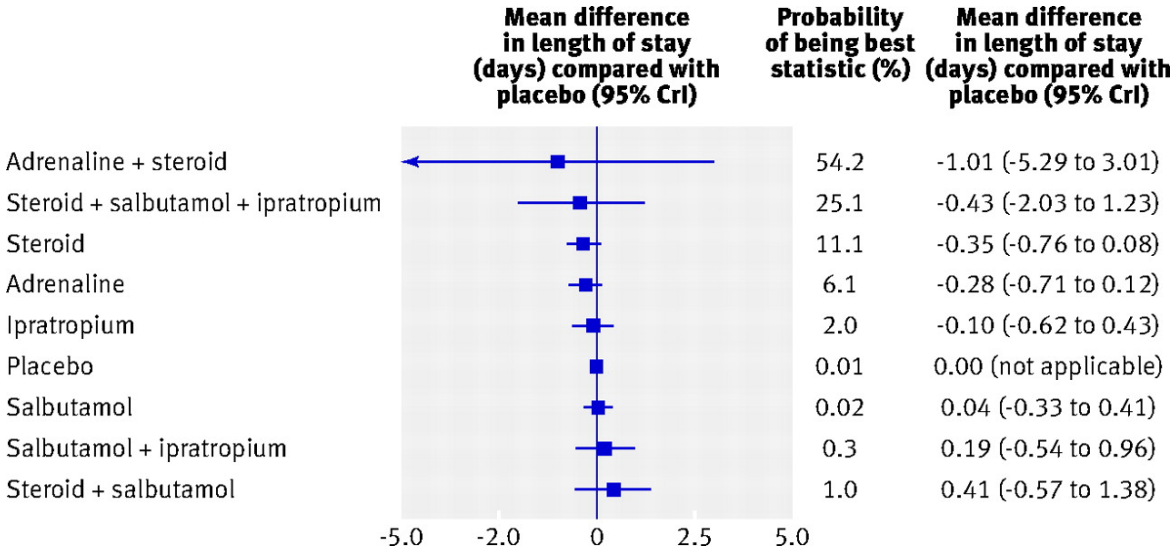


Figure 2.7. Results of mixed treatment analysis for length of stay, showing probability ranking and probability of being best statistic.

combined with dexamethasone showed longer term effects, reducing admission rates up to seven days after the emergency department visit. The strength of evidence for this reduction in admission rates was considered low based on the GRADE system, largely because it came from a single trial; however, recent empirical evidence suggests that reliance on evidence from a single precise trial that has been carried out well is reasonable.[758] The effectiveness of these interventions is supported by positive benefits in some of the secondary outcome measures, including short term changes in clinical score, with no observed concerns about short term safety. For inpatients, none of the interventions examined showed clear benefits for length of stay. Adrenaline showed some significant improvements for short term changes in clinical score, whereas steroids and salbutamol showed benefits com- pared with placebo over the longer follow-up periods (3-6 and 6-12 hours).

Interpretation of findings

The magnitude and timing of observed effects of adrenaline and steroids are supported through their known mechanisms of actions. Adrenaline has been shown to improve short term clinical variables, but its effect on admission rates was unclear, mostly due to underpowered studies.[759] We did not confirm concerns that the early benefit shown might result in a later increase in admissions or return visits.[541] The longstanding claim that the α adrenergic vasoconstriction and edema reducing activity of adrenaline may confer advantage over β adrenergic only drugs was supported by direct and indirect comparisons suggesting some benefit over salbutamol.[760] Findings also indicate that previous conflicting results on the use of steroids may be partially explained by interaction with bronchodilators. Our analysis clearly excludes a clinically relevant stand alone effect of steroids but shows additive effects when combining a long action steroid such as dexamethasone with use of bronchodilators that follow a protocol. It is recognized that the immune response plays a significant part in the pathogenesis of bronchiolitis, although the biological action of anti-inflammatory interventions may be limited.[761,762] Clinical synergism between steroids and bronchodilators is a major topic in the long term treatment of asthma and chronic obstructive pulmonary disease.[763] Findings from translational research show a two way molecular interaction between these drugs, including β2 agonist stimulated steroid mediated gene transcription, and a steroid induced increase in the transcription of

the β2 receptor gene.[650] Whether these mechanisms are involved in the treatment of acute bronchiolitis, and the contribution of specific types and doses of bronchodilators and steroids, is unknown.

Another outstanding problem is the difference in observed effects between inpatient and outpatient populations. Adrenaline shows benefits for outpatients but not for inpatients. This may be attributable to short term compared with long term response or characteristics of the patients (for example, responders vs non- responders) or illness (for example, timing and severity of infection).

Safety concerns exist about the widespread use of adrenaline and steroids in young children with viral wheezing, particularly with repeated high doses.[647,764] Our results do not suggest any serious or frequent short term expected or unexpected harms from any of the studied interventions in infants with bronchiolitis in the absence of comorbidities. However, our safety analysis is based on randomized trials, which often have limited power to detect important differences owing to the infrequent occurrence of events. Data from trials and observational studies in croup confirm a favorable short term safety profile.[601] Long term problems raised by the use of steroids in prematurity include effects on adrenal function, cardio- vascular responses, somatic and lung growth, and neurodevelopment.[765-769] Evidence is, however, scarce on the effects for short term use in otherwise healthy term infants, and none of these were studied in included trials.

Limitations of existing evidence

Our strength of evidence assessments provide clarity around the limitations of this body of evidence and direction for future research. Two key factors affected the strength of evidence: potential risk of bias in the included studies and sparsity of data for many of the outcomes and comparisons, which resulted in imprecise estimates and unknown consistency of estimates across studies. Risk of bias was high due to potential selective outcome reporting, incomplete outcome data, and lack of blinding. Reporting of sequence generation and allocation concealment was often unclear. Sparsity of data was a result of few studies making the same comparisons as well as variability in the choice of outcomes and timing of outcome assessments. The message around consistency and relevance of outcomes is not

new for this discipline.[589,770] Further work to define clinically important efficacy and safety outcomes for bronchiolitis is ongoing.

Implications for research

Future research should focus on areas where there is some suggestion of benefit (significant or close to significant results in direct meta-analysis with a magnitude of effect that is clinically meaningful, or relative superiority in mixed treatment comparisons) but the strength of evidence is moderate or low—that is, future research may change our confidence in the estimate and may change the estimate. Based on this review, adrenaline and combined adrenaline and dexamethasone seem to be emerging as the preferred treatments for outpatients. This review found no clear advantage of steroids or bronchodilators among inpatients. This information should guide the choice of comparators, including their dose and combinations, for future large trials.

Strengths and limitations of the review

This review has followed current methodological standards for the synthesis of evidence. Moreover, we have incorporated new methods of analysis to simultaneously compare the different interventions and to provide greater clarity around their relative benefits. Limitations of mixed treatment comparisons have been cited, specifically assumptions of sufficient homogeneity to combine data and generalizability to individual patients.[661] The influence of age, history of wheezing episodes, and wheezing phenotype has led to repeated controversies in this subject, and it is not yet clear how best to approach these problems at a trial and systematic review level.[771,780] We focused on first time wheezing so results could be directly pertinent to infants with typical viral bronchiolitis. We searched extensively for relevant literature and included all studies regardless of language of publication. We are confident that this review represents the most comprehensive synthesis currently available for the two most promising treatments for bronchiolitis, steroids and bronchodilators.

Conclusion

Uncertainty about the optimal management of bronchiolitis is underscored by evidence showing substantial variation in practice, even within homogenous clinical settings. Current clinical practice guidelines recommend only supportive

measures based on the absence of convincing evidence for any other approach. This systematic review shows a benefit of adrenaline for outcomes of most clinical relevance among outpatients. Moreover, adrenaline is shown to be safe and is relatively inexpensive. Some evidence exists for a beneficial synergistic effect of adrenaline and dexamethasone. Further research of this combined treatment is needed among outpatients. For inpatients, none of the interventions examined showed clear benefits for length of stay. Consensus on the most clinically important outcomes and consistency in their application will yield stronger evidence for this important source of morbidity among young children.

2.2 CORTICOSTEROIDS FOR ACUTE VIRAL BRONCHIOLITIS IN INFANTS AND YOUNG CHILDREN

Adapted from:

- Fernandes RM, Bialy LM, Vandermeer B, Tjosvold L, Plint AC, Patel H, Johnson DW, Klassen TP, Hartling L. Glucocorticoids for acute viral bronchiolitis in infants and young children. Cochrane Database of Systematic Reviews 2010, Issue 10. Art. No.: CD004878. DOI: 10.1002/14651858.CD004878.pub3.

- Fernandes RM, Bialy LM, Vandermeer B, Tjosvold L, Plint AC, Patel H, Johnson DW, Klassen TP, Hartling L. Glucocorticoids for acute viral bronchiolitis in infants and young children. Cochrane Database of Systematic Reviews 2013, Issue 6. Art. No.: CD004878. DOI: 10.1002/14651858.CD004878.pub4.
  [updated version of the 2010 review]

- Fernandes RM, Hartling L. Glucocorticoids for acute viral bronchiolitis in infants and young children. Journal of the American Medical Association. 2014;311:87-8.
  [summary of the 2013 Cochrane review for JAMA's Clinical Evidence Synthesis]

A list of included studies is shown in the Appendix A2 of this thesis.

Authors

Ricardo M Fernandes (1), Liza M Bialy (2), Ben Vandermeer (2), Lisa Tjosvold (3), Amy C Plint (4), Hema Patel (5), David W Johnson (6), Terry P Klassen (7), Lisa Hartling (2)

Affiliations

1. Laboratory of Clinical Pharmacology and Therapeutics, Faculty of Medicine, University of Lisbon, Instituto de Medicina Molecular; and Department of Pediatrics, Santa Maria Hospital, Lisboa
2. Department of Pediatrics, University of Alberta, Edmonton, Canada

3. Alberta Research Centre for Child Health Evidence, University of Alberta, Edmonton
4. Departments of Pediatrics and Emergency Medicine, University of Ottawa, Ottawa
5. Department of Pediatrics, The Montreal Children's Hospital, Montreal
6. Department of Pediatrics, Faculty of Medicine, University of Calgary, Alberta Children's Hospital, Calgary
7. Manitoba Institute of Child Health, Winnipeg

ABSTRACT

Background

Previous systematic reviews have not shown clear benefit of glucocorticoids for acute viral bronchiolitis, but their use remains considerable. Recent large trials add substantially to current evidence and suggest novel glucocorticoid-including treatment approaches.

Objectives

To review the efficacy and safety of systemic and inhaled glucocorticoids in children with acute viral bronchiolitis.

Search methods

We searched the Cochrane Central Register of Controlled Trials (CENTRAL 2012, Issue 12), MEDLINE (1950 to January week 2, 2013), EMBASE (1980 to January 2013), LILACS (1982 to January 2013), Scopus® (1823 to January 2013) and IRAN MedEx (1998 to November 2009).

Selection criteria

Randomized controlled trials (RCTs) comparing short-term systemic or inhaled glucocorticoids versus placebo or another intervention in children under 24 months with acute bronchiolitis (first episode with wheezing). Our primary outcomes were: admissions by days 1 and 7 for outpatient studies; and length of stay (LOS) for inpatient studies. Secondary outcomes included clinical severity parameters, healthcare use, pulmonary function, symptoms, quality of life and harms.

Data collection and analysis

Two authors independently extracted data on study and participant characteristics, interventions and outcomes. We assessed risk of bias and graded strength of evidence. We meta-analyzed inpatient and outpatient results separately using random-effects models. We pre-specified subgroup analyses, including the combined use of bronchodilators used in a protocol.

Main results

We included 17 trials (2596 participants); three had low overall risk of bias. Baseline severity, glucocorticoid schemes, comparators and outcomes were heterogeneous. Glucocorticoids did not significantly reduce outpatient admissions by days 1 and 7 when compared to placebo (pooled risk ratios (RRs) 0.92; 95% confidence interval (CI) 0.78 to 1.08 and 0.86; 95% CI 0.7 to 1.06, respectively). There was no benefit in LOS for inpatients (mean difference -0.18 days; 95% CI -0.39 to 0.04). Unadjusted results from a large factorial low risk of bias RCT found combined high-dose systemic dexamethasone and inhaled epinephrine reduced admissions by day 7 (baseline risk of admission 26%; RR 0.65; 95% CI 0.44 to 0.95; number needed to treat 11; 95% CI 7 to 76), with no differences in short-term adverse effects. No other comparisons showed relevant differences in primary outcomes.

Authors' conclusions

Current evidence does not support a clinically relevant effect of systemic or inhaled glucocorticoids on admissions or length of hospitalization. Combined dexamethasone and epinephrine may reduce outpatient admissions, but results are exploratory and safety data limited. Future research should further assess the efficacy, harms and applicability of combined therapy.

BACKGROUND

Description of the condition

Acute viral bronchiolitis is the most common acute infection of the lower respiratory tract during the first year of life.[1] It is diagnosed clinically in infants and young children, based on a history of rhinorrhoea and low-grade fever that progress to cough and respiratory distress, with findings of tachypnoea, chest retractions and

wheeze, crackles, or both, on examination.[2,3] RSV is responsible for the majority of cases, usually in seasonal epidemics.[2,772] Other viral agents, particularly rhinovirus, human metapneumovirus, bocavirus and adenovirus, may also be involved as single or dual infections.[5,119,123,124] Although bronchiolitis is usually a straightforward diagnosis, some variability in its definition exists. This may be due to poor agreement on the identification of early childhood wheezing phenotypes and worldwide differences in disease semantics.[4,214,460]

Bronchiolitis is a major cause of clinical morbidity and its financial health burden is substantial. Population-based studies in developed countries suggest an incidence ratio of approximately 10% within the first year of life, with hospital admissions up to 3%.[1,37,47,81] While mortality is rare, hospitalizations have increased steadily in North America and Europe over the past 10 to 20 years, with rising inpatient health care costs.[37,64,67,69,70,73] Additionally, a majority of cases with mild illness cared for in the community are responsible for a considerable number of outpatient visits, loss of parental work time and decreased quality of life.[53,82,451] RSV infection, including bronchiolitis, is a major cause of childhood morbidity and mortality at a global level.[38]

Bronchiolitis involves acute inflammation of the bronchiolar airways initiated by viral infection, regardless of the causative agent. Airway edema, necrosis and mucous plugging are the hallmark pathological features, and air flow obstruction ensues. Factors underlying disease severity are only partially understood, but clinical determinants include lower age, prematurity, chronic lung, heart or neurological disease, immunodeficiency and ethnicity.[278,283,351,773] There is likely a complex interplay between host (i.e. genetic markers), agent (i.e. viral loads, specific agents and co-infections) and environmental factors (i.e. crowding, tobacco smoke exposure).[5,6,91,212,287,290] Basic, translational and clinical research studies are elucidating the association between bronchiolitis, preschool wheezing disorders and later asthma.[7,48,458,774]

Description of the intervention

The current treatment for bronchiolitis is controversial. There is substantial variation in its management throughout the world, reflecting the absence of clear evidence for any single treatment approach.[8,9,44,81,549,553] Many interventions failed to show

consistent and relevant effects.[10] Recently, both nebulized epinephrine and hypertonic saline have emerged as options for improving relevant outcomes in outpatient and inpatient populations, respectively.[775,776] However, no routine treatment is yet recommended by most evidence-based clinical practice guidelines worldwide.[393,394, 743]

The case of glucocorticoids highlights the uncertainties of research in this field. Trials assessing their use date back to the 1960s, with different potencies, modes of administration, dosages and regimens of these drugs having been recommended.[578,585] However, results from RCTs have been heterogeneous, leading to ongoing controversy regarding their use. Differences in participants, care settings and outcomes may account for these conflicting results, and have led to distinct interpretations.[4,590,728,781,777]

How the intervention might work

Glucocorticoid use in bronchiolitis was originally thought to have equivalent benefits to those in acute asthma. Similarities between clinical findings were expected to express equivalent biological and physiological mechanisms attributable to inflammation.[578] However, evidence suggests there is heterogeneity in inflammatory pathways and mediators activated in different wheezing phenotypes which may underlie bronchiolitis (for example, neutrophil- versus eosinophil-mediated inflammation).[778] Mechanistic studies have shown that glucocorticoids have limited anti-inflammatory effects in this condition and there is an ongoing debate regarding their efficacy in acute virus-induced wheezing in preschool children.[643,647,648,649,779] Further, potential benefits need to be considered in light of possible short- and long-term adverse effects of glucocorticoid use. While the interactive effect of bronchodilators and glucocorticoids has been widely known in asthma, both at a clinical and biological level, its use as a putative treatment option in bronchiolitis has only been explored recently.[46]

Why it is important to do this review

While guideline implementation has changed prescription patterns, glucocorticoids are still widely used.[551,552,780] The latest version of this review integrated critical results from the two largest multi-centre studies in this area and examined the use of combined therapy with bronchodilators or adrenaline.[45,46] We continue to

update the current body of evidence in order to adequately assess the efficacy and safety of glucocorticoids in bronchiolitis.

OBJECTIVES

To review the efficacy and safety of systemic and inhaled glucocorticoids in children with acute viral bronchiolitis.

METHODS

Criteria for considering studies for this review

Types of studies

We included RCTs irrespective of risk of bias, sample size, publication status or language of publication.

Types of participants

Studies should include infants and young children ≤ 24 months of age with acute viral bronchiolitis. Bronchiolitis was defined clinically as a first episode of acute wheezing, respiratory distress and clinical evidence of a viral infection (cough, coryza, fever). Many bronchiolitis trial reports do not specify clinical findings required for participant inclusion; we included all studies if other diagnoses (for example, pneumonia) could be excluded.[589] We did not restrict inclusion based on specific findings on examination (for example, crackles) or viral etiology.

We excluded studies in which any participant had a history of wheezing or respiratory distress (one or more previous episodes), a formal diagnosis of asthma, or if reporting of these items was unclear. We focused on first time wheezing so results could be directly pertinent to infants with 'typical' viral bronchiolitis, as opposed to children with acute recurrent wheezing. We did not exclude trials based on other reported participant characteristics, including gestational age and co-morbidities.

We included studies of both inpatients and outpatients (ambulatory care and/or emergency department), and excluded trials in the intensive care setting or with intubated and/or ventilated participants.

Types of interventions

The interventions of interest were short-term systemic or inhaled glucocorticoids administered for the acute care of bronchiolitis. We considered all types of glucocorticoids, dosages, durations and routes of administration. Glucocorticoids could be administered alone or combined with co-interventions (for example, bronchodilators), used with or without a fixed protocol. We excluded trials assessing the use of longer courses of glucocorticoids started during the acute phase for the prevention of post-bronchiolitic wheezing.

Comparators included either placebo or another intervention (for example, bronchodilators, other glucocorticoid). Inhaled isotonic saline is frequently used as a placebo control for inhaled drugs. We excluded studies comparing different doses or regimens of the same glucocorticoid.

Types of outcome measures

We selected primary outcomes based a priori on clinical relevance and patient importance; secondary outcomes assessed other relevant health domains (clinical severity, pulmonary function, healthcare use, patient/parent-reported symptoms and status, and harms). We included studies if they reported numeric data on at least one primary or secondary outcomes assessed within the first month after acute bronchiolitis. We considered different timings of outcome assessment, based on a priori relevance and available data.

Primary outcomes

1. Rate of admission by days one and seven for outpatient studies.
2. Length of stay (LOS) for inpatient studies.

Secondary outcomes

1. Clinical severity scores.
2. O2 saturation, respiratory rate and heart rate.
3. Hospital re-admissions (for inpatient studies) and return healthcare visits (for all studies); LOS (for outpatient studies)
4. Pulmonary function tests.
5. Symptoms and quality of life.
6. Short- and long-term adverse events.

We selected the following time points and intervals for clinical scores, O2 saturation, respiratory and heart rate: 60 and 120 minutes, three to six hours, six to 12 hours, 12 to 24 hours, 24 to 72 hours, and three to 10 days. The time points selected for re-admissions and return visits were days 1 to 10, and 11 to 30. We also considered data on all other reported outcomes.

Search methods for identification of studies

The previous version of this review used an inclusive search strategy as part of a comprehensive systematic review evaluating the effect of three types of interventions in bronchiolitis (glucocorticoids, epinephrine and other bronchodilators) (Chapter 2).

Electronic searches

Previously we searched the Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library 2009, Issue 4), which contains the Cochrane Acute Respiratory Infections Group's Specialised Register, MEDLINE (1950 to November Week 2, 2009), EMBASE (1980 to Week 47, 2009), LILACS (Latin American and Caribbean Center on Health Sciences Information) (1982 to 25 November 2009), Scopus® (1823 to 25 November 2009) and IRAN MedEx (1998 to 26 November 2009). We developed search strings by scanning search strategies of relevant systematic reviews and examining index terms of potentially relevant studies. We applied and modified a validated RCT filter according to each database (Glanville 2006). We applied no publication or language restrictions.

For this 2013 update we searched the Cochrane Central Register of Controlled Trials (CENTRAL) 2012, Issue 12, part of The Cochrane Library, www.thecochranelibrary.com (accessed 21 January 2013), which contains the Cochrane Acute Respiratory Infections Group's Specialised Register, MEDLINE (October 2009 to January week 2, 2013), EMBASE (November 2009 to January 2013), LILACS (Latin American and Caribbean Center on Health Sciences Information) (2009 to January 2013) and Scopus (2009 to January 2013). Search strategies were adapted from those presented in Appendix A1.

Searching other resources

To identify unpublished studies and studies in progress we searched the following clinical trials registers on 1 August 2012: ClinicalTrials.gov and ICTRP Search Portal - World Health Organization. We searched the following conference proceedings: Pediatric Academic Societies (2003 to 2012), European Respiratory Society (2003 to 2011), American Thoracic Society (2006 to 2012). We identified additional published, unpublished or ongoing studies by hand-searching reference lists and included or excluded studies of relevant reviews. In addition, we contacted topic specialists.

Data collection and analysis

Selection of studies

Five review authors (AP, LB, LH, NH or RF) independently screened the titles, keywords and abstracts (when available) to determine if an article met the inclusion criteria. These review authors independently assessed the full text of all articles classified as 'include' or unclear' using a standardized form. We resolved disagreements by consensus or by an arbitrator (AP, TK, DJ, or RF).

Data extraction and management

We extracted data using a standardized form in paper or electronic format (available from authors). Seven review authors extracted data (LB, LH, AM, HM, RF, OT or JF) and three review authors (LB, AM or RF) independently checked for accuracy and completeness. We resolved discrepancies by consensus or in consultation with a third review author (TK, AP or DJ). A statistician (BV) checked all quantitative data during analysis. Extracted data included study characteristics, funding, inclusion/exclusion criteria, participant characteristics, interventions, outcomes and results.

Assessment of risk of bias in included studies

We used the Cochrane 'Risk of bias' assessment tool, which includes seven domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other sources of bias.[749] We assessed blinding and incomplete outcome data separately for the following groups of outcomes: healthcare use (rate of admission, LOS, hospital re-admissions and return healthcare visits); clinical parameters (clinical severity scores, O2 saturation,

respiratory rate and heart rate); pulmonary function; patient/parent-reported outcomes (symptoms and quality of life measures) and other outcomes such as adverse events. Where trial protocols or trial registers were unavailable, we assessed selective outcome reporting by comparing outcomes reported in the methods and results sections. We summarized risk of bias for each study across outcomes based on individual domain assessments ('high' if one or more domains were high; 'low' if all domains were low; 'unclear' for all other studies). Three review authors (LB, LH or RF) independently assessed the risk of bias of the included studies; we resolved discrepancies by consensus. One review author (OT) assessed study reports written in Turkish. We pilot tested the risk of bias tool on a sample of five studies and used the results to adapt decision rules (available from authors).

Grading the body of evidence

We used the Evidence-Based Practice Centers GRADE approach, based on the standard GRADE system (GRADE 2009; Owens 2010), to assess domain-specific and overall strength of evidence on three relevant outcomes: length of stay or admission rate, clinical severity scores and adverse events. Two review authors (LH, RF) independently graded the body of evidence using adapted decision rules.

We examined the following domains: risk of bias, consistency, directness and precision. Risk of bias was considered as low or medium, as we only included RCTs. There is limited evidence regarding clinically significant and patient-important between- group differences in this field. We therefore defined a priori thresholds of clinical relevance based on expert opinion and GRADE guidance for the precision domain: risk ratio reduction > 20% for admissions, reduction in LOS > 0.5 days and clinical scale effect sizes based on GRADE guidance (GRADE 2009). We graded overall strength of evidence 'high', 'moderate' or 'low' based on the likelihood of further research changing our confidence in the estimate of effect (when evidence was unavailable or did not permit estimation of an effect, it was considered insufficient). All decisions were made explicitly and inter-rater agreement was calculated (data available from authors). We resolved discrepancies by consensus among two review authors (LH, RF).

Measures of treatment effect

We pooled dichotomous variables using risk ratios (RRs). We derived the number needed to treat to benefit (NNTB) for significant results from primary outcomes. Since the only comparison with significant differences was based on a single trial, the NNTB is shown for that trial's baseline risk.

We analyzed measurement scale outcomes as continuous variables. For continuous variables measured on the same scale (for example, respiratory rate), we calculated mean differences (MD) for individual studies and mean differences for the pooled estimates. For those measured on different scales (for example, clinical scores), we calculated MDs for separate studies and standardized MD (SMD) for the pooled estimates. We used changes from baseline for all continuous variables.

Unit of analysis issues

Some of the studies included in this review were multi-arm or factorial studies in which more than two intervention groups were eligible to contribute several comparisons to a single meta-analysis. For example, a trial might compare glucocorticoid versus placebo in two arms, and glucocorticoid + bronchodilator versus placebo + bronchodilator in another two arms, with both contributing to the overall glucocorticoid versus placebo comparison. When the comparisons were independent, i.e. with no intervention group in common, we included data from these arms with no transformation and we shown them separately in each forest plot. If needed and feasible, we pooled the active groups to avoid double-counting of the comparator group when there was more than one active group: for example, two glucocorticoid groups versus placebo. We did not include any treatment groups twice in the same meta-analysis.

Guidance regarding the analysis of factorial trials mandates caution when results suggest positive interaction/additive effects ('synergism') between study treatments.[658,781] This was the case for a large trial included in this review. We therefore chose to include comparisons separately in meta-analysis ('within the table analysis'): for example, for the glucocorticoid versus placebo comparison, we included separately glucocorticoid + bronchodilator versus placebo + bronchodilator and glucocorticoid + placebo versus double placebo. We also performed sensitivity analysis pooling all arms ('at the margins analysis').

Dealing with missing data

We extracted information on incomplete outcome data and we classified trials that performed intention-to-treat (ITT) analysis as either ITT with all data, ITT with imputation of missing data, ITT with available case analysis, per protocol analysis or treatment-received analysis.[749] We did not impute missing data for drop-outs. We estimated unreported means from figures or imputed from medians if possible. We computed standard deviations (SDs) from available data (i.e. standard errors, confidence intervals (CI) or P values) when missing. Failing this, we estimated them from ranges and inter-quartile ranges, or imputed them from a similar study. When standard deviations of change from baseline values were unavailable, we estimated correlation at 0.5.[782,783] We occasionally encountered clinical score results presented as dichotomous data, for example, using a cut-off score or time-to-event analysis. When methods were feasible and assumptions judged reasonable, we used existing approaches to re-express odds ratios as standardized mean differences, thus allowing dichotomous and continuous data to be pooled together.[749] When data were unavailable for one of the predefined timings of outcome measurement, we used the time point closest or any time point in the range. If there was more than one time point, we chose the one with the largest magnitude of change. We did not contact trial authors of the individual studies to obtain additional data.

Assessment of heterogeneity

We quantified statistical heterogeneity using the I^2 statistic. We used the following intervals for interpreting I^2 statistic values: 0% to 30% low heterogeneity; 30% to 50% moderate heterogeneity; 50% to 75% substantial heterogeneity; and 75% to 100% considerable heterogeneity.[749]

Assessment of reporting biases

We assessed reporting biases for the main comparisons and primary outcomes by visual interpretation of funnel plots and testing for funnel plot asymmetry (Egger test).[749]

Data synthesis

We meta-analyzed quantitative results within the different comparisons when studies were consistent on clinical grounds and had available outcome data; we

imposed no restrictions based on risk of bias. We performed separate meta-analyses for studies involving inpatients and outpatients. We combined results using random-effects models regardless of heterogeneity, due to expected differences in interventions, outcomes and measurement instruments. We calculated fixed-effect models in a sensitivity analysis. We conducted meta-analyses of dichotomous outcomes using Mantel-Haenszel methods. We used inverse variance methods for continuous outcomes and measurement scales, and combined dichotomous and continuous data into a standardized mean difference whenever needed.[749] All results are reported with 95% CI. We used Review Manager software for data management and analysis (RevMan 2012).

Subgroup analysis and investigation of heterogeneity

We planned to investigate heterogeneity by conducting subgroup analyses based on pre-specified study- and participant-level characteristics. The following subgroups were considered:

1. Protocolized use of bronchodilators (studies with protocolized use versus no/unclear protocolized use).
2. RSV status (studies with all participants exclusively RSV- positive versus some RSV-negative/unspecified RSV status).
3. Age of participants (studies with all participants exclusively less than 12 months of age versus some participants older than 12 months/unspecified age).
4. Atopy (studies with all participants exclusively atopic versus some participants not atopic/unspecified atopic status).
5. Glucocorticoid: type of glucocorticoids; and daily and overall dose (high versus low).

We explored potential positive or negative (i.e. 'synergistic' or 'antagonistic') interactions between glucocorticoids and bronchodilators by distinguishing trials where bronchodilator use was protocolized (i.e. comparing glucocorticoids + bronchodilator versus placebo + bronchodilator) from studies where use was either at the discretion of the physician or not allowed.[657] The choice of RSV, age and atopy was based on clinical or biological evidence suggesting possible effect modification of glucocorticoid effects by these parameters. We studied drug type and dose to explore distinct glucocorticoid pharmacokinetic and

pharmacodynamic properties; dosing was based on prednisolone equivalents. We planned to perform subgroup analyses only on the review's primary outcomes. We also collected data from studies that analyzed these subgroups at a study level. We assessed subgroup differences comparing changes in effect estimate and CI overlap; statistical tests or meta-regression techniques were not used.

Sensitivity analysis

We decided a priori to perform sensitivity analyses on primary outcome results of trials with overall low risk of bias. We also checked for differences in the direction and magnitude of primary outcome results when using fixed-effect models, as well as using pooled data from all factorial trial arms ('at the margins analysis').

RESULTS

Description of studies

Results of the search

The initial 2009 comprehensive search of all electronic databases identified 2249 records, of which 344 were potentially relevant. Hand-searching had identified four more studies and overall 348 full-text articles had been assessed for eligibility. Of 91 studies that used glucocorticoids, 17 trials fulfilled inclusion criteria. The 2013 search identified 280 further records, of which 13 were assessed for eligibility using full text but all were excluded (flowchart in Figure 2.8).

Included studies

We included 17 trials with 2596 randomized participants. We considered different comparisons separately between glucocorticoids, alone or with fixed co-interventions, and either placebo or active controls. Included trials contributed to one or more comparisons, depending on trial arms (Figure 2.8).

Design, centers and sample sizes

Fifteen trials were parallel-designed, 14 of which were double-armed (Bentur 2005; Berger 1998; Cade 2000; Corneli 2007; De Boeck 1997; Goebel 2000; Gomez 2007; Klassen 1997; Mesquita 2009; Richter 1998; Roosevelt 1996; Schuh 2002; Teeratakulpisarn 2007; Zhang 2003) and one was six-armed (Barlas 1998).Two trials were factorial two-by-two (Kuyucu2004; Plint 2009). Eleven trials were single-centred and five included multiple centres (range: 2 to 20) (Cade 2000; Corneli

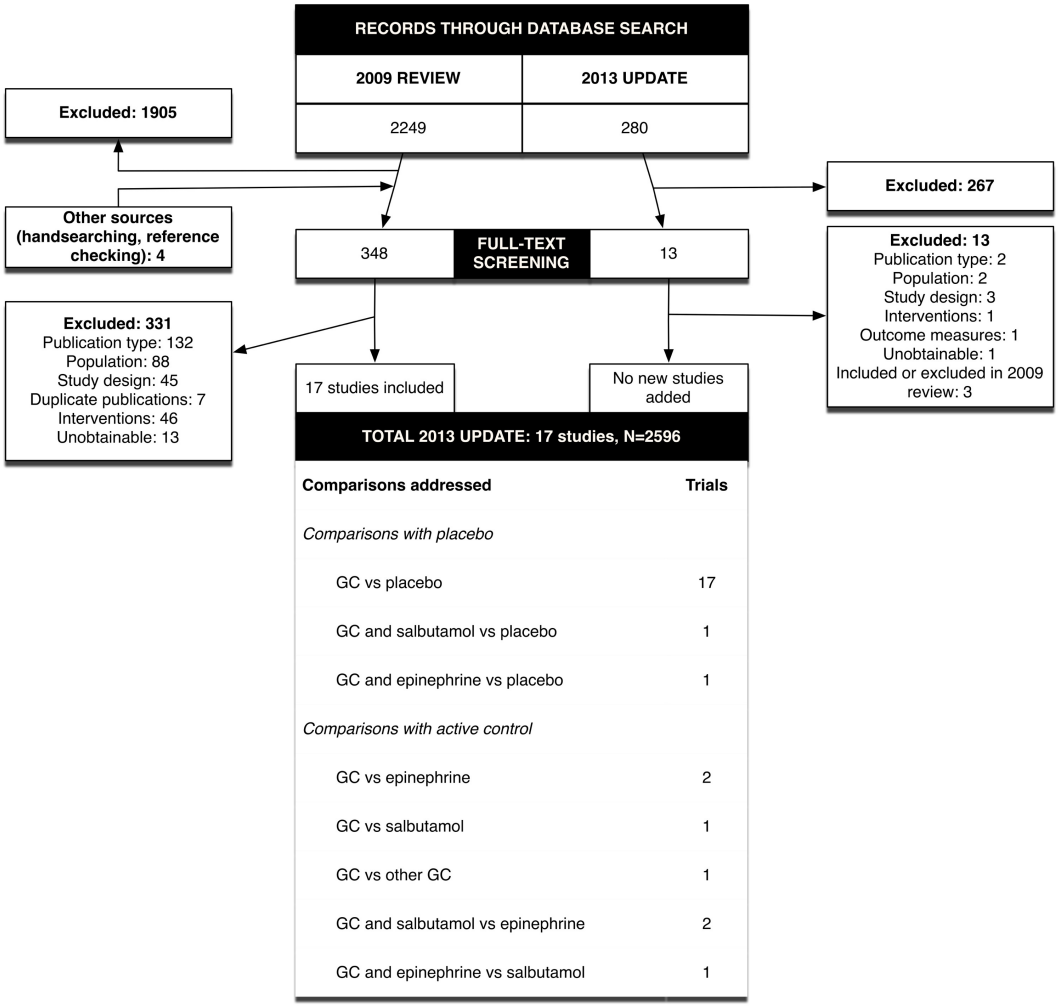


Figure 2.8. Flow of citations through the search and screening procedures of the 2009 review and this 2012 update, studies included in the review and comparisons addressed (GC: glucocorticoids)

2007; Goebel 2000; Plint 2009; Teeratakulpisarn 2007); one trial did not clearly report this item (Bentur 2005). All trials were conducted in a single country, either in North, Central or South America, Europe and the Middle East or Asia.

Sample size calculations were reported in 12 trials (Bentur 2005; Berger 1998; Cade 2000; Corneli 2007; Klassen 1997; Mesquita 2009; Plint 2009; Richter 1998; Roosevelt 1996; Schuh 2002; Teeratakulpisarn2007; Zhang2003); the outcome used for sample size calculation was the reported primary outcome in all except one trial (Richter 1998). The overall median number of participants per trial was 72 (range 32 to 800), with two large trials counting 600 and 800 (Corneli 2007; Plint 2009,

respectively), and all others fewer than 200. Funding was reported in nine studies, three of which had pharmaceutical industry support (Cade 2000; Richter 1998; Schuh 2002).

Setting and participants

Outpatients were included in eight trials, with 1824 randomized participants and a median of 85 participants per trial (range: 42 to 800) (Barlas 1998; Berger 1998; Corneli 2007; Goebel 2000; Kuyucu 2004; Mesquita 2009; Plint 2009; Schuh 2002). Outpatient settings mostly included paediatric emergency departments. Nine trials included inpatients only, with 772 participants and a median of 61 participants per trial (range: 32 to 179) (Bentur 2005; Cade 2000; De Boeck 1997; Gomez 2007; Klassen 1997; Richter 1998; Roosevelt 1996; Teeratakulpisarn 2007; Zhang 2003). Few details were reported regarding criteria for hospitalization and the type of admission unit in which patients received care, except for one inpatient trial report (Teeratakulpisarn 2007). In most trials bronchiolitis was defined by clinical findings; wheezing was always required. Three trials restricted inclu- sion to bronchodilator responders (Goebel 2000 - outpatients; Teeratakulpisarn 2007 and Zhang 2003 - inpatients). Seven trials only included participants under the age of 12 months, all of which had a mean or median participant age below six months (Bentur 2005; Cade 2000; Corneli 2007; Plint 2009; Richter 1998; Roosevelt 1996; Zhang 2003).

Bronchiolitis severity thresholds were used for inclusion in eight outpatient (Barlas 1998; Berger 1998; Corneli 2007; Goebel 2000; Kuyucu 2004; Mesquita 2009; Plint 2009; Schuh 2002) and two inpatient trials (Gomez 2007; Klassen 1997). Severity was based on clinical scales or respiratory parameters, and thresholds varied. The Respiratory Distress Assessment Instrument (RDAI) baseline score thresholds varied between two and six (less than four usually considered mild bronchiolitis).

Thirteen trials reported testing for RSV at least in a portion of participants, and three trials only included RSV-positive patients (Bentur 2005; Cade 2000; De Boeck 1997). Prevalence of RSV in the remaining 10 trials varied from 33% to 89% (Barlas 1998; Berger 1998; Corneli 2007; Goebel 2000; Klassen 1997; Mesquita 2009; Plint 2009; Richter 1998; Roosevelt 1996; Schuh 2002). Atopic status was reported

in nine trials (Barlas 1998; Berger 1998; Cade 2000; Plint 2009; Richter 1998; Roosevelt 1996; Schuh 2002; Teeratakulpisarn 2007; Zhang 2003), while one trial reported a family history of wheezing (Corneli 2007). Definitions for atopy and methods of assessment were rarely provided, and when reported were heterogeneous. No trials excluded participants with a history of atopy.

Children with chronic cardiac, pulmonary or neurological conditions or immunodeficiency were frequently excluded. All or some premature infants were explicitly excluded in seven trials (Cade 2000; Corneli 2007; De Boeck 1997; Goebel 2000; Plint 2009; Schuh 2002; Teeratakulpisarn 2007). Other criteria for exclusion were length of illness and glucocorticoid-related parameters (previous use, history of adverse events, specific contraindications to their use).

Subgroup analyses within studies were reported in five trials (Bentur 2005; Cade 2000; Corneli 2007; Plint 2009; Teeratakulpisarn 2007), two of which being pre-specified (Corneli 2007; Plint 2009). Subgroups were based on age, RSV status, family or personal history of atopy and eczema, duration and severity of illness, and exposure to smoke and/or dampness.

Interventions

There was heterogeneity regarding the choice of glucocorticoid, its dosage, route of administration and duration of treatment. Dexamethasone was the most frequently tested drug (11 trials). Nine trials used systemic dexamethasone, either oral (Corneli 2007; Klassen 1997; Mesquita 2009; Plint 2009; Schuh 2002), intramuscular (Kuyucu 2004; Roosevelt 1996; Teeratakulpisarn 2007) or intravenous (De Boeck 1997). Single-day doses were administered for one to five days. Initial dosing was higher (0.5 to 1 mg/kg), with later doses ranging from 0.15 to 0.6 mg/kg. The highest overall dose was seen in Plint 2009 and Schuh 2002 (1 mg/kg followed by 0.6 mg/kg for five days), and the lowest in Mesquita 2009 (single-dose 0.5 mg/kg). Two trials used inhaled dexamethasone (0.2 mg to 0.25 mg every four to six hours), at least for one day, or until discharge for inpatients (Bentur 2005; Gomez 2007). Systemic prednisone or prednisolone were tested in four trials, three oral (Berger 1998; Goebel 2000; Zhang 2003) and one intravenous (Barlas 1998). Duration varied between one and five days (1 to 2 mg/kg/day, once or twice daily). Three trials used inhaled budesonide (0.5 mg to 1 mg, once or twice daily) for one to six

weeks (Barlas 1998; Cade 2000; Richter 1998). Details on placebos were reported in nine trials. Inhaled placebos included mist (Barlas 1998) and 0.9% saline (Bentur 2005; Richter 1998). Protocolized standard of care was used as a control arm in Zhang 2003.

Eleven trials used protocolized bronchodilators in both glucocorticoid and placebo arms. The choice of bronchodilator, its dose and frequency varied substantially. Seven trials used salbutamol (Barlas 1998; Berger 1998; Goebel 2000; Gomez 2007; Klassen 1997; Kuyucu 2004; Schuh 2002), four used epinephrine (Bentur 2005; Kuyucu 2004; Mesquita 2009; Plint 2009) and one used salbutamol and ipratropium bromide (De Boeck 1997). Nebulized salbutamol was administered during emergency department stay (first two to four hours), or each four to six hours at home or during hospitalization (1.5 mg to 2.5 mg, or 0.15 mg/kg). Oral administration was also allowed in Goebel 2000. Nebulized epinephrine was administered every six hours to inpatients, or once or twice in the emergency department for outpatients (1 mg to 3 mg). All other trials used bronchodilators at the discretion of the attending physician, often with guidance on the choice of drug and dosage. Additional use of glucocorticoids was often restricted. Supportive measures, i.e. oxygen and intravenous or nasogastric fluids, were usually reported.

Outcomes

Pre-defined primary outcomes were specified in 12 trials (Cade 2000; Corneli 2007; Goebel 2000; Klassen 1997; Kuyucu 2004; Mesquita 2009; Plint 2009; Richter 1998; Roosevelt 1996; Schuh 2002; Teeratakulpisarn 2007; Zhang 2003), three of which reported more than one primary outcome (Kuyucu 2004; Richter 1998; Teeratakulpisarn 2007). Only the two largest trials used admission as a primary outcome (Corneli 2007; Plint 2009). Other primary outcomes included clinical scales (Goebel 2000; Klassen 1997; Kuyucu 2004; Mesquita 2009; Richter 1998; Schuh 2002), clinical severity parameters or duration of disease (Kuyucu 2004; Roosevelt 1996; Teeratakulpisarn 2007) and symptoms (Cade 2000; Zhang 2003). Timings of primary outcome assessment were reported in 11 trials, six of which used multiple time points. Sample size calculations were either not reported or based on secondary outcomes in Goebel 2000, Kuyucu 2004 and Richter 1998. Reported outcomes included healthcare use domains and clinical severity parameters (all trials), pulmonary function (De Boeck 1997), patient/parent-reported

symptoms and status (seven trials: Berger 1998; Cade 2000; Plint 2009; Roosevelt 1996; Schuh 2002; Teeratakulpisarn 2007; Zhang 2003) and other outcomes, including adverse events (10 trials: Bentur 2005; Cade 2000; Corneli 2007; Klassen 1997; Kuyucu 2004; Plint 2009; Richter 1998; Roosevelt 1996; Teeratakulpisarn 2007; Zhang 2003). Not all outcome and time point results were reported.

Admission rates were assessed in all eight outpatient trials, both by day 1 (all trials) and day 7 (three trials; Corneli 2007; Plint 2009; Schuh 2002). Kuyucu 2004 and Goebel 2000 reported admissions by days 5 and 6, respectively, and were pooled with day 7 results. LOS was reported in eight of nine inpatient trials (except Roosevelt 1996) and three outpatient trials (Berger 1998; Corneli 2007; Goebel 2000). Criteria for admission or discharge were rarely reported. Considerable variability was found in control group admission rates (from 0% to 44% by day 1, and 0% to 49% by day 7) and mean LOS (0.8 to 6.6 days) (Table 2.4). Hospital re-admissions for inpatients and return healthcare visits up to one month were mentioned in six trials, with variable assessment methods (Berger 1998; Klassen 1997; Plint 2009; Roosevelt 1996; Schuh 2002; Teeratakulpisarn 2007).

Clinical severity scales were assessed in all except one trial (Zhang 2003), often using more than one scale (Corneli 2007; Plint 2009; Richter 1998; Schuh 2002). Measurement instruments were developed specifically for nine trials (Barlas 1998; Bentur 2005; Berger 1998; Cade 2000; De Boeck 1997; Goebel 2000; Richter 1998; Roosevelt 1996; Teeratakulpisarn 2007), mostly based on previous scales by Schuh 1990, Tal 1983 and Westley 1978. The RDAI was used in eight trials (Corneli 2007; Gomez 2007; Klassen 1997; Kuyucu 2004; Mesquita 2009; Plint 2009; Richter 1998; Schuh 2002). Corneli 2007 and Plint 2009 also used the Respiratory Assessment Change Score (RACS), based on RDAI and respiratory rate (both originally reported by Lowell 1987). All scales included items on wheezing and accessory muscle use; other respiratory items (for example, timing or location of wheezing) or disease domains (for example, general status, nutrition) were less frequently used. Oxygen saturation, respiratory and heart rates were reportedly measured in most trials. Heterogeneity in timings of repeated measurements was found; the two most frequently time points assessed were 60 minutes and three to six hours. Measurement of patient/parent-reported symptoms was inconsistent. Five trials reported symptoms data (Cade 2000; Plint 2009; Richter 1998; Roosevelt

Table 2.4: Placebo group risk of admission/length of stay*

Study	Placebo group - participants	Placebo group - primary outcomes	
Outpatient studies		**Risk of admission day 1 (%)**	**Risk of admission day 7 (%)**
Barlas 1998	30	17%	NR
Berger 1998	18	11%	NR
Corneli 2007	295	41%	49%
Goebel 2000	24	8%	21%
Kuyucu 2004	11	0%	0%
Mesquita 2009	32	22%	NR
Plint 2009	201	18%	26%
Schuh 2002	34	44%	47%
Inpatient studies		**Length of stay (mean ± SD days)**	
Bentur 2005	32	6.3 ± 8.8	
Cade 2000	79	2 ± 2.2	
De Boeck 1997	15	6.6 ± 1.2	
Gomez 2007	25	0.8 ± 0.2	
Klassen 1997	32	2 ± 0.7	
Richter 1998	19	3 ± 1.6	
Teeratakulpisarn 2007	85	2.8 ± 1.7	
Zhang 2003	24	5 ± 3.3	

*NR = not reported; SD = standard deviation

1996; Teeratakulpisarn 2007). There were differences in the specific symptoms addressed (for example, respiratory, feeding), the measurement instrument used (i.e. questionnaires, diaries) and the time points of assessment. No trial reported the use of generic or disease-specific quality of life instruments.

Other reported outcomes included temperature measurements (Corneli 2007; Plint 2009; Roosevelt 1996), time to resolution or length of illness (Roosevelt 1996; Zhang 2003), and duration of oxygen therapy or fluids (Bentur 2005; Richter 1998; Roosevelt 1996; Teeratakulpisarn 2007; Zhang 2003). Data on the use of bronchodilator co-interventions were often reported as an outcome.

Adverse events were mentioned in six trials (Corneli 2007; Goebel 2000; Klassen 1997; Kuyucu 2004; Plint 2009; Teeratakulpisarn 2007). Five of these studies assessed specific gastrointestinal, endocrine or infectious complications. There was heterogeneity and incomplete reporting regarding which adverse events were pre-specified, their definitions and measurement methods. All adverse effects were short-term and no study assessed long-term harms.

Excluded studies

Eighty-four out of 361 excluded papers involved glucocorticoids. Motives for exclusion from this subset mostly included inappropriate population (for example, trials including participants with a history of previous wheezing, or > 24 months old), type of publication and non-RCT study design.

Risk of bias in included studies

We assessed overall risk of bias as 'low' in three trials, as 'high' in seven and 'unclear' in seven. The glucocorticoid and epinephrine versus placebo comparison included one low risk of bias trial. All other comparisons included mostly high risk of bias trials (Figure 2.9).

We found adequate sequence generation and allocation concealment in 10 and 11 trials, respectively (Figure 2.10). We considered blinding adequate in 10 out of 17 trials for the review primary outcomes and clinical severity parameters. Incomplete reporting explained most 'unclear' assessments. Incomplete outcome data were adequately addressed in 12 out of 17 studies for the review primary outcomes, and 11 out of 17 for clinical severity outcomes; it was unclear or inadequate when there was imbalanced attrition between groups, mostly in longer follow-up assessments.

We considered nine out of 17 studies free from risk of selective outcome reporting. Assessment of this item was challenging given the large number of outcomes reported, the diversity of measurement time points, and the fact that trial protocols were not available. Using trial registry searches, we identified three trial registers and used that data to complete assessments (Corneli 2007; Plint 2009; Teeratakulpisarn 2007).

Figure 2.9. Methodological quality graph: review authors' judgements about each methodological quality item presented as percentages across all included studies. [For multi-arm studies (Barlas 1998, Kuyucu 2004 and Plint 2009), we included one overall assessment for all trial comparisons, and two assessments for each separate comparison of glucocorticoids versus placebo (with or without protocolised bronchodilator, or with epinephrine or salbutamol]

Regarding publication bias and small study effects, there was no asymmetry in funnel plots for the primary outcomes in the glucocorticoids versus placebo comparison by visual inspection or statistical testing (Egger test for admissions and length of stay, P = 0.98 and P = 0.77, respectively) (Figure 2.11; Figure 2.12).

Other types of bias assessed as 'unclear' included baseline imbalances, or active arm contamination with other related co-interventions (Kuyucu 2004 and Schuh 2002, respectively).

Effects of interventions

Results are summarized by comparison, setting and type of outcome. GRADE assessments for the two main comparisons - glucocorticoid versus placebo and glucocorticoid and bronchodilator versus placebo are shown in Table 2.5 and Table 2.6. All meta-analyses used random-effects models; fixed-effect models did not modify the direction and magnitude of results unless mentioned.

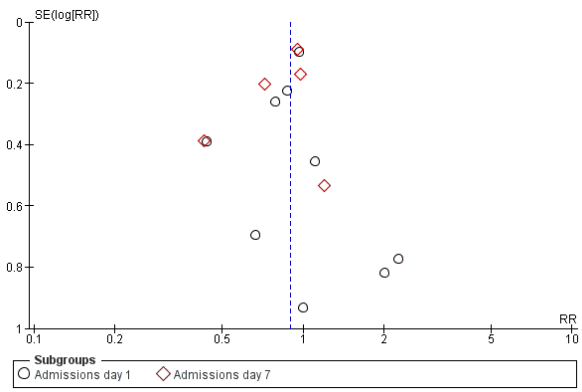


Figure 2.11. Funnel plot of comparison: 1 Steroid versus placebo, outcome: 1.1 Admissions (days 1 and 7) (outpatients) - review primary outcome.

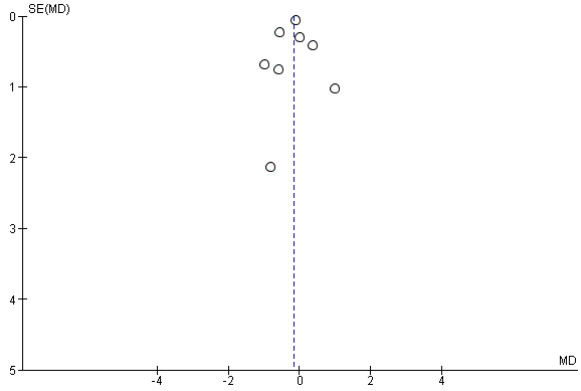


Figure 2.12. Funnel plot of comparison: 1 Steroid versus placebo, outcome: 1.2 Length of stay (inpatients) - review primary outcome.

Summary of findings for the glucocorticoid versus placebo comparison

Glucocorticoid versus placebo for acute viral bronchiolitis in infants and young children

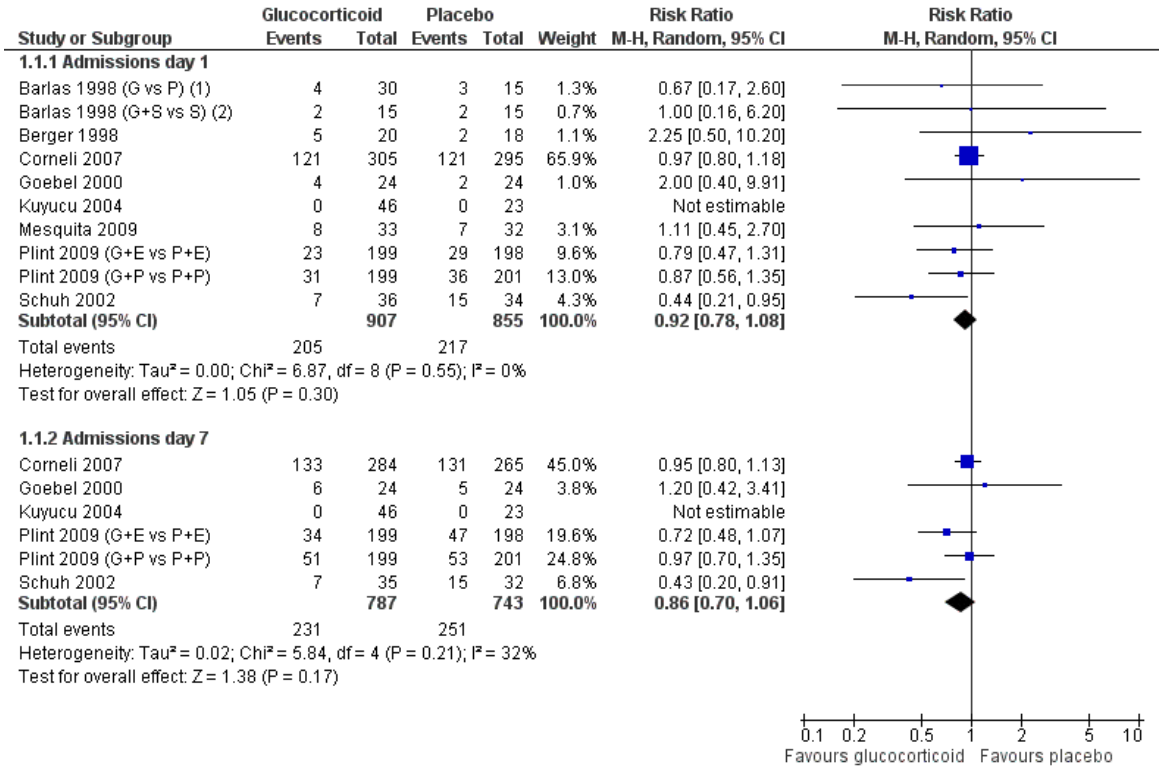
Patient or population: infants and young children with acute viral bronchiolitis
Settings: outpatients and inpatients
Intervention: glucocorticoid versus placebo

Outcomes	Illustrative comparative risks* (95% CI)		Steroid versus placebo	No of participants (studies)	Quality of the evidence (GRADE)
	Assumed risk[1]	Corresponding risk			
	Placebo	Steroid			
Admissions (outpatients) Follow-up: day 1	**Medium risk population**		**RR 0.92** (0.78 to 1.08)	1762 (8)	high
	162 per 1000	**149 per 1000** (126 to 175)			
Admissions (outpatients) Follow-up: day 7	**Medium risk population**		**RR 0.86** (0.7 to 1.06)	1530 (5)	moderate
	250 per 1000	**215 per 1000** (175 to 265)			
Length of stay (inpatients) days	The mean length of stay ranged across control groups from 0.8 to 6.6 days	The mean length of stay in the intervention groups was **0.18 lower** (0.39 lower to 0.04 higher)		633 (8)	high

*The basis for the **assumed risk** (for example, the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI)
CI: confidence interval
RR: risk ratio

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Assumed risk for admissions was based on the median control group risks across the studies included in the meta-analysis (medium risk).



(1) Plint 2009 (factorial trial) and Barlas 1998 (parallel multiarm study) contribute two independent comparisons which are shown separately;
(2) G: Glucocorticoid, S:Salbutamol, E: Epinephrine, P: Placebo

Figure 2.13. Forest plot of comparison: 1 Steroid versus placebo, outcome: 1.1 Admissions (days 1 and 7) (outpatients) - review primary outcome.

Glucocorticoid versus placebo

Outpatients

Primary outcomes

All eight outpatient studies reported admissions by day 1, and five also reported admissions by day 7. Complete outcome data were available for 1762 participants by day 1 (out of 1824 randomized) and 1530 participants by day 7 (out of 1612 randomized).

The pooled RRs for admissions by days 1 and 7 were 0.92 (95% confidence interval (CI) 0.78 to 1.08) and 0.86 (95% CI 0.7 to 1.06), respectively, with no significant differences between groups (Analysis 1.1; Figure 2.13). Heterogeneity was low for day 1 results and moderate for day 7 (I^2 statistic = 0% and 31%, respectively). There was no relevant change in the magnitude or direction of results when using pooled data from both Plint 2009 arms. Sensitivity analyses for both trials with low overall risk of bias showed comparable results (Analysis 1.22). Overall strength of evidence

Figure 2.10. Methodological quality summary: review authors' judgements about each methodological quality item for each included study. [For multi-arm studies (Barlas 1998, Kuyucu 2004 and Plint 2009), we included one overall assessment for all trial comparisons, and two assessments for each separate comparison of glucocorticoids versus placebo (with or without protocolized bronchodilator, or with epinephrine or salbutamol)]

for these findings was high for day 1 results and moderate for day 7, the latter due to some imprecision in the effect estimate (Table 2.5; Summary of findings for the main comparison).

Subgroup analysis of studies using protocolized bronchodilator found lower pooled RRs for admissions by both days 1 and 7, but the CIs between subgroups overlapped (Analysis 1.15; Analysis 1.16). For admissions by day 7, the estimate for RR was 0.68 (95% CI 0.44 to 1.05) for protocolized bronchodilator trials (four trials, 581 participants), and 0.95 (95% CI 0.82 to 1.11) for other trials (two trials, 949 participants). Heterogeneity was low in both subgroups.

The two largest outpatient studies only included participants under 12 months of age, while six smaller studies also included older patients (Analysis 1.17; Analysis 1.18). For admissions by day 7, estimates were 0.92 (95% CI 0.80 to 1.06) and 0.67 (95% CI 0.25 to 1.83), for < 12 months (two trials, 1346 participants) and trials including older participants (three trials, 184 participants), respectively. Trials including older participants had a lower effect estimate, but a large CI overlapped with the other subgroup and there was substantial heterogeneity (I^2 statistic = 60%).

No subgroup analysis according to respiratory syncytial virus (RSV) or atopic status was performed, since no outpatient trial restricted inclusion based on these parameters. Corneli 2007 and Plint 2009 reported pre-specified subgroup analyses based on atopic status, with no statistically significant differences. Plint 2009 also reported no differences according to RSV status, duration of illness and severity. We chose not to perform analyses based on glucocorticoid type or dose due to heterogeneity in glucocorticoid schemes.

Secondary outcomes

Clinical score data were available for time points/intervals between 60 minutes and 3 to 10 days (Analysis 1.4; Figure 2.14). Different sets of studies with different scales contributed to each time point, with most data at 60 minutes (four trials, 1006 participants); no trial assessed the period between 24 to 72 hours. There were no significant differences between groups at any time point. Strength of evidence for these findings was high at 60 minutes, with precise and consistent results (SMD

-0.04; 95% CI -0.16 to 0.09; I^2 statistic = 0%). Evidence was weaker for later results due to imprecision and substantial heterogeneity.

Six trials reported outcome data on oxygen saturation between 60 minutes and 24 to 72 hours (Analysis 1.6). Data were most frequently reported at 60 minutes (three trials, 936 participants). At three to six hours, results favored placebo (MD -0.43; 95% CI -0.84 to -0.02; units: %), while for all other time points there were no significant differences between groups. Respiratory and heart rate data were both reported in six outpatient trials, between 60 minutes and 3 to 10 days (Analysis 1.8; Analysis 1.10). The most frequently assessed time point for both outcomes was 60 minutes; no trial assessed the period between 24 to 72 hours. There were no significant differences between groups for any of these outcomes.

Regarding other health services outcomes, pooled data from three trials (255 participants) reporting LOS of admitted patients did not show significant differences between groups (Analysis 1.3). Return to healthcare visits for bronchiolitis symptoms were only assessed in two trials (863 participants), both showing considerable event rate for a three to four-week follow-up period (26% to 53% in all groups; Table 2.7). Pooled results did not show significant differences between groups (RR 1.04; 95% CI 0.80 to 1.35) (Analysis 1.14).

Plint 2009 reported data on parent-reported symptoms regarding time to return to normal feeding, sleeping, breathing and no coughing (Table 2.8). There were no statistically significant differences between glucocorticoid and placebo groups. No outpatient trials assessed or reported pulmonary function or quality of life outcomes.

Inpatients

Primary outcomes

Eight inpatient trials reported data on LOS (633 participants), with no significant mean difference between glucocorticoid and placebo groups (MD -0.18 days; 95% CI -0.39 to 0.04; I^2 statistic = 16%) (Analysis 1.2; Figure 2.15). On a sensitivity analysis using fixed-effect models and including all studies, the mean difference reached statistical significance favoring glucocorticoids, with a similar magnitude (MD -0.14 days; 95% CI -0.25 to -0.03). We graded the strength of evidence as high

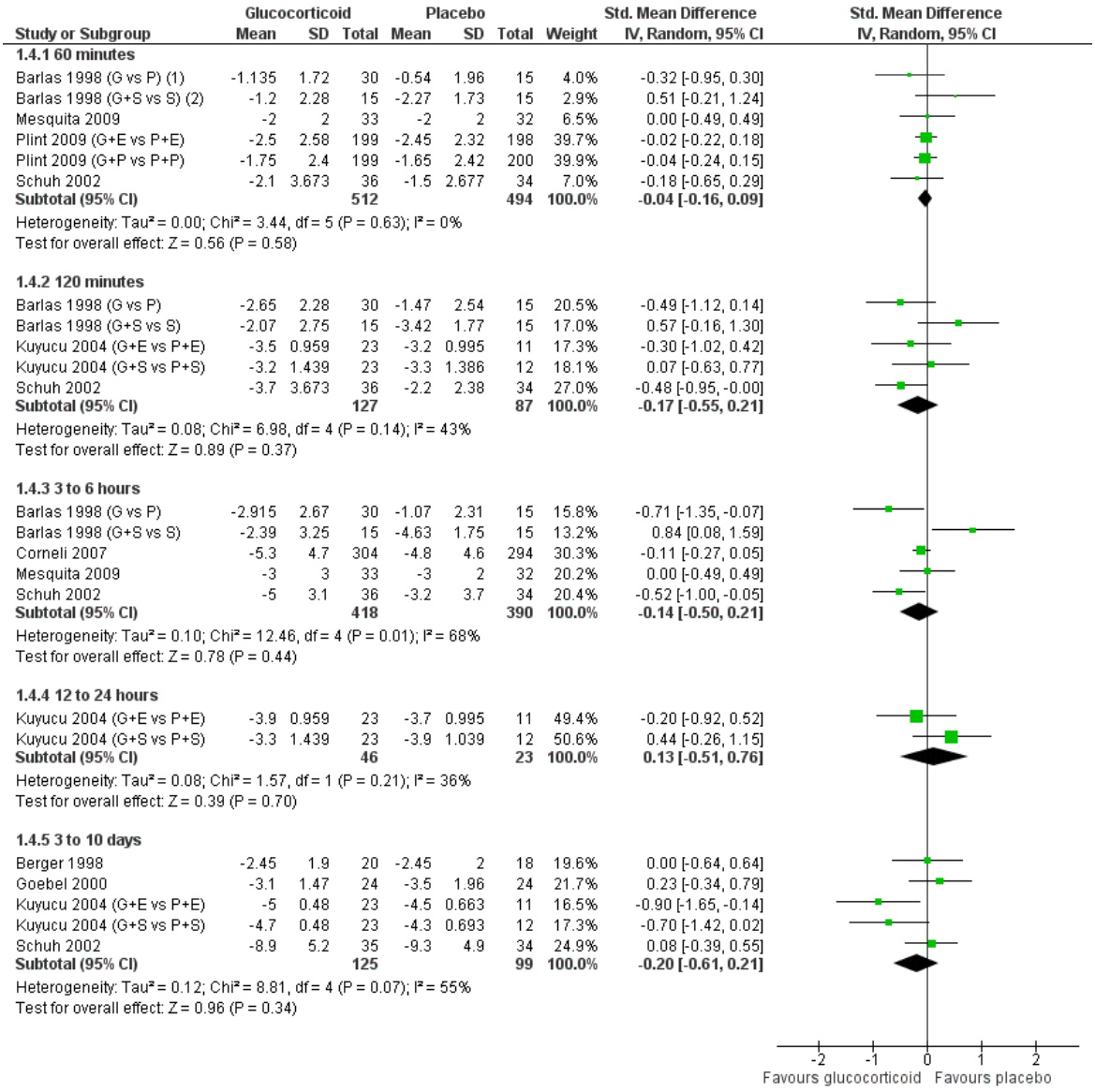


Figure 2.14. Forest plot of comparison: 1 Steroid versus placebo, outcome: 1.4 Clinical scale scores (outpatients) (change from baseline data).

given its precision, consistency and 'Risk of bias' assessments for all included trials (Table 2.5; Summary of findings for the main comparison).

Subgroup analyses showed a statistically significant reduction in LOS in trials with protocolized bronchodilator (-0.12 days; 95% CI -0.23 to -0.00; four trials, 206 participants), although CIs overlapped between subgroups (Analysis 1.19).

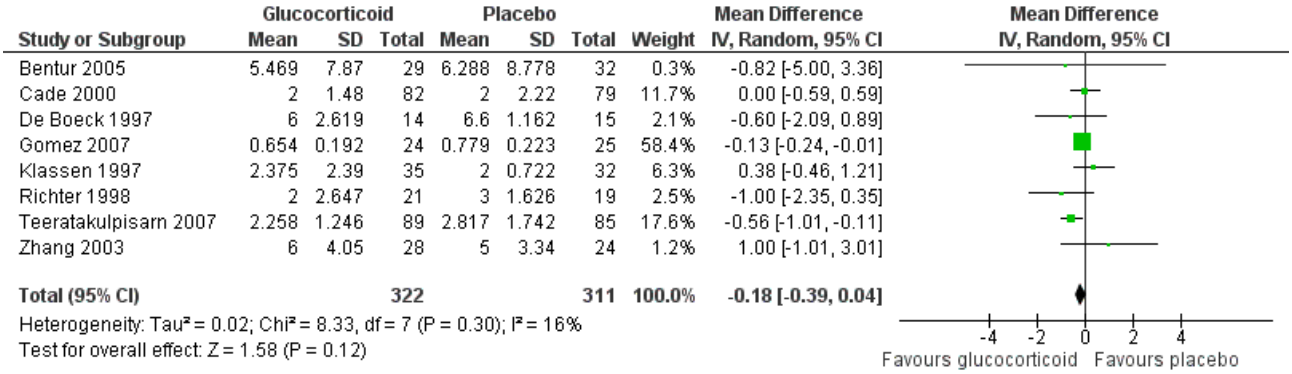


Figure 2.15. Forest plot of comparison: 1 Steroid versus placebo, outcome: 1.2 Length of stay (inpatients) - review primary outcome.

Heterogeneity was low in the protocolized group results (I^2 statistic = 0%) and moderate in the other subgroup (I^2 statistic = 38%). In subgroup analyses according to age and RSV status, CIs overlapped between subgroups for both parameters (Analysis 1.20 and Analysis 1.21). Heterogeneity was low in both < 12 months and RSV-only trial results, and moderate in the other subgroups. We did not perform subgroup analyses based on atopic status and glucocorticoid type and dose for the reasons mentioned previously.

Secondary outcomes

Clinical score data were only available for intervals between three to six hours and 24 to 72 hours (Analysis 1.5; Figure 2.16). Glucocorticoids were favored at earlier time points (three to six hours, one trial, 174 participants: SMD -1.03 (95% CI -1.87 to -0.19); and 6 to 12 hours, three trials, 269 participants: SMD -0.62 (95% CI -1.00 to -0.23). There were no statistically significant differences at later time points. We assessed the overall strength of evidence for these findings as low or moderate, due to imprecision and low or unknown consistency, often with considerable heterogeneity.

Only two trials reported outcomes of oxygen saturation and respiratory rate at time points between 6 to 12 hours and 24 to 72 hours, one of which also reported heart rate at 12 to 24 hours (Analysis 1.7; Analysis 1.9; Analysis 1.11). There were no significant differences between groups for any outcome or time point. Both hospital re-admissions and return healthcare visits were reported by three inpatient studies, with distinct durations of follow- up; no significant differences were found between groups (Table 2.7; Analysis 1.12; Analysis 1.13).

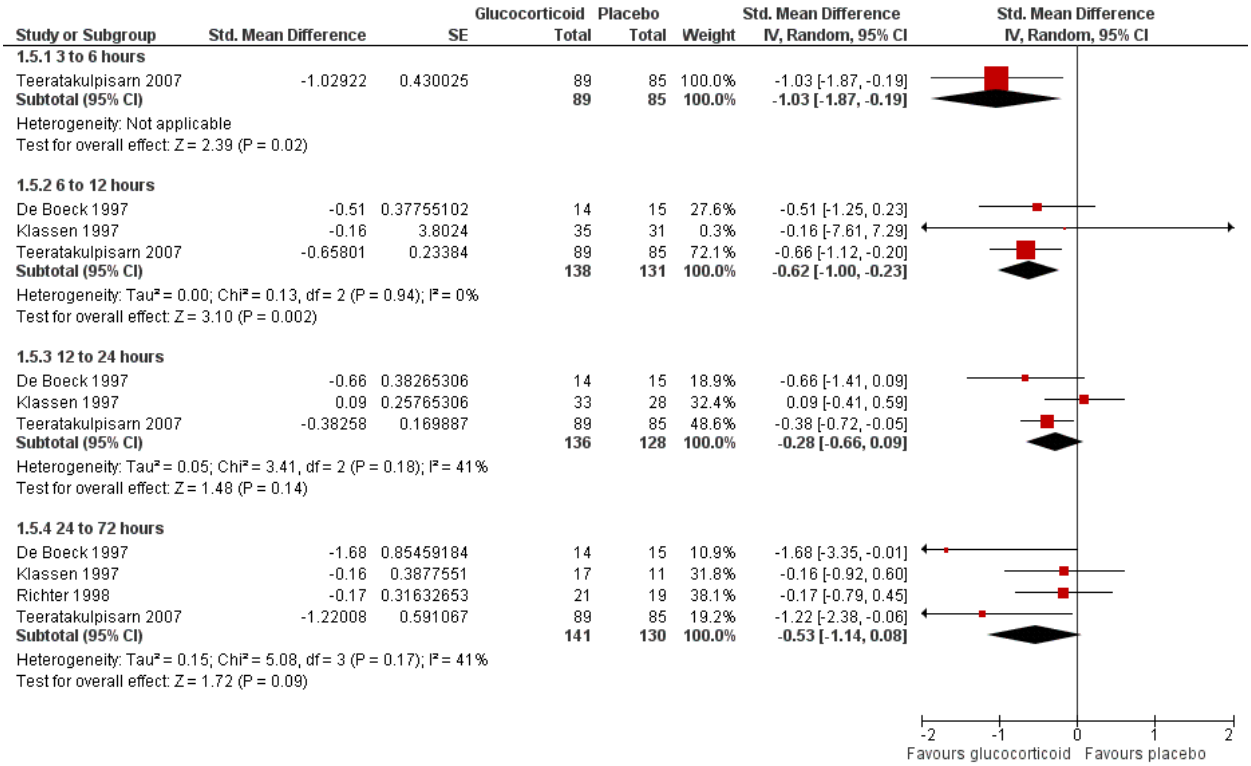


Figure 2.16. Forest plot of comparison: 1 Glucocorticoid versus placebo, outcome: 1.6 Clinical scores (inpatients) (change from baseline data).

Three inpatient trials reported data on parent-reported symptoms (Table 2.8). Different sets of symptoms were measured at distinct time points, and methods of measurement and analysis varied. In Teeratakulpisarn 2007 time to being symptom free was significantly shorter in the glucocorticoid group, while Cade 2000 used a different analysis and did not shown any statistically significant differences. There were no differences regarding respiratory symptoms and feeding in both Cade 2000 and Roosevelt 1996. No inpatient trials assessed or reported quality of life outcomes.

De Boeck 1997 reported results from pulmonary function tests on day three. No differences were found in minute ventilation, dynamic lung compliance, and inspiratory and expiratory pulmonary resistance, both before and after nebulised bronchodilator.

All patients

Adverse events

Six trials reported adverse events. Five assessed specific glucocorticoid-related harms including the two largest studies (Table 2.9). We considered all harms data together regardless of patient setting in order to adequately assess the safety profile of glucocorticoids. Data were available from 600 to 1579 participants for each safety outcome. We did not pool results given the heterogeneity in definitions, methods and timings of assessment. Individual trial analysis did not show significant differences between glucocorticoids and placebo regarding the occurrence of vomiting, gastrointestinal bleeding, hypertension, pneumonia or varicella.

Table 2.5: GRADE assessments: glucocorticoid versus placebo

Population	Outcome	Nr of studies	Nr of participants	GRADE domains				Strength of evidence	Intervention favoured
				Risk of bias	Consistency	Directness	Precision		
GLUCOCORTICOID versus PLACEBO									
Inpatient	Length of stay	8	633	Medium	Consistent	Direct	Precise	High	No difference
	Clinical score : 3 to 6 hours	1	26	Medium	Unknown	Direct	Imprecise	Low	Glucocorticoid
	Clinical score : 6 to 12 hours	3	175	Medium	Consistent	Direct	Imprecise	Moderate	Glucocorticoid
	Clinical score : 12 to 24 hours	3	230	Medium	Consistent	Direct	Imprecise	Moderate	No difference (glucocorticoid favoured)
	Clinical score : 24 to 72 hours	4	113	Medium	Inconsistent	Direct	Imprecise	Low	No difference (glucocorticoid favoured; very close to significant)
Outpatient	Admissions day 1	8	1762	Medium	Consistent	Direct	Precise	High	No difference
	Admissions up to day 7	5	1530	Low	Consistent	Direct	Imprecise	Moderate	No difference
	Clinical score: 60 minutes	4	1006	Low	Consistent	Direct	Precise	High	No difference

Table 2.5: GRADE assessments: glucocorticoid versus placebo

	Clinical score: 120 minutes	3	214	Medium	Consistent	Direct	Imprecise	Moderate	No difference
	Clinical score: 3 to 6 hours	2	808	Medium	Inconsistent	Direct	Precise	Moderate	No difference
	Clinical score: 12 to 24 hours	1	69	Medium	Unknown	Direct	Imprecise	Low	No difference
	Clinical score: 3 to 10 days	4	224	Medium	Inconsistent	Direct	Imprecise	Low	No difference
Inpatient/ outpatient	Adverse events	5	1123	Low	Consistent	Direct	Precise	Moderate	No difference

Table 2.6: GRADE assessments: glucocorticoid and epinephrine versus placebo*

Population	Outcome	Nr of studies	Nr of participants	GRADE domains				Strength of evidence	Intervention favoured
				Risk of bias	Consistency	Directness	Precision		
GLUCOCORTICOID AND EPINEPHRINE versus PLACEBO									
Outpatient	Admissions day 1	1	400	Low	Unknown	Direct	Imprecise	Low	Favours epi + dex but NS
	Admissions up to day 7	1	400	Low	Unknown	Direct	Imprecise	Low	Epi + dex
	Clinical score: 60 minutes	1	400	Low	Unknown	Direct	Precise	Moderate	Epi + dex
	Adverse events	1	400	Low	Unknown	Direct	Imprecise	Low	No difference

*dex = dexamethasone; epi = epinephrine; NS = non-significant

Summary of findings for the glucocorticoid and epinephrine versus placebo comparison

Glucocorticoid and epinephrine versus placebo for acute viral bronchiolitis in infants and young children

Patient or population: infants and young children with acute viral bronchiolitis
Settings: outpatients
Intervention: glucocorticoid and epinephrine versus placebo

Outcomes	Illustrative comparative risks* (95% CI)		Steroid versus placebo	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk[1]	Corresponding risk				
	Placebo	Steroid				
Admissions (outpatients) Follow-up: day 1	**179 per 1000**	**115 per 1000** (72 to 186)	**RR 0.65** (0.4 to 1.05)	400 (1)	Low	NNT: not calculated for non-significant findings
Admissions (outpatients) Follow-up: day 7	**264 per 1000**	**169 per 1000** (116 to 251)	**RR 0.65** (0.44 to 0.95)	400 (1)	Low	NNT: 11 (95% CI 7 to 76) (based on unadjusted analysis results)

*The basis for the **assumed risk** (for example, the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI)
CI: confidence interval
NNT: number needed to treat
RR: risk ratio

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Assumed risk for admissions was based on the control group risk in the single study included (Plint 2009).

Glucocorticoid and bronchodilator (epinephrine or salbutamol) versus placebo

Both outpatient trials assessing either of these comparisons used different severity thresholds for patient inclusion: Respiratory Distress Assessment Instrument (RDAI) score above four in Plint 2009 (moderate disease), and scores between 4 and 10 using a trial-specific clinical scale in Barlas 1998 (mild to moderate disease).

Primary outcomes

The factorial trial Plint 2009 included a comparison of oral dexamethasone and nebulized epinephrine against double placebo (399 analyzed participants). This was the largest trial included in the review, with low overall risk of bias. The RRs for admissions by days 1 and 7 were 0.65 (95% CI 0.40 to 1.05) and 0.65 (95% CI 0.44 to 0.95), respectively (Analysis 2.1). There was a statistically significant reduction in admissions by day 7, with a relative risk reduction estimate of 35%. Absolute risk reduction was 9% (95% CI 1 to 17), and the number needed to treat to benefit (NNTB) to reduce one admission by day 7 was 11 (95% CI 7 to 76); these results were obtained through unadjusted analysis. However, the factorial trial design requires special methodological considerations, since this was not the

study's main comparison, and there was an unanticipated additive/synergistic effect between epinephrine and dexamethasone. Reported analyses adjusted for multiple comparisons were above the threshold for statistical significance (RR 0.65; 95% CI 0.41 to 1.03). We graded the overall strength of evidence as low for these results given their imprecision and the fact that they were obtained from a single trial (Table 2.6; Summary of findings 2).

Barlas 1998, a small high risk of bias trial, compared intravenous prednisolone and nebulized salbutamol versus placebo. Admissions by day 1 (30 participants) showed no statistically significant differences between groups (RR 0.67; 95% CI 0.13 to 3.44) (Analysis 3.1).

Secondary outcomes

Clinical score results at 60 minutes favored glucocorticoid and epinephrine (SMD -0.34; 95% CI -0.54 to -0.14) (Analysis 2.2), while having an increased heart rate (MD 8.44; 95% CI 4.85 to 12.03) (Analysis 2.5). No differences were found between groups regarding oxygen saturation and respiratory rate (Analysis 2.3; Analysis 2.4). There were also no differences regarding return healthcare visits for bronchiolitis symptoms (RR 1.11; 95% CI 0.89 to 1.38) (Table 2.7; Analysis 2.6). Symptom results showed reduced time to normal feeding and quiet breathing in the glucocorticoid and epinephrine group (mean symptom duration ratios: 0.63; 95% CI 0.5 to 0.8 and 0.83; 95% CI 0.69 to 1.00) (Table 2.8). No differences were found in time to normal sleeping and time to no coughing.

Results for clinical scores, oxygen saturation and heart rate at 60 minutes, 120 minutes and three to six hours did not show any differences between groups in the single trial comparing glucocorticoid and salbutamol versus placebo (Analysis 3.2; Analysis 3.3; Analysis 3.4). No further secondary outcomes were assessed in this comparison.

<u>Other comparisons</u>

These included glucocorticoid versus bronchodilator (epinephrine or salbutamol), glucocorticoid and bronchodilator (epinephrine or salbutamol) versus different bronchodilator (epinephrine or salbutamol), and direct comparisons between different types of glucocorticoid (prednisolone versus budesonide). All trials were

performed in the outpatient setting, and all except one were small-sized and had a high risk of bias.

Primary outcomes

The glucocorticoid versus epinephrine comparison included data from two trials (444 participants) for admissions by day 1, and one trial by day 7 (399 participants). Risk of bias was low for one trial and high for the other. There were no significant differences between groups at both time points (Analysis 4.1). Only one small high risk of bias trial included data on day 1 admissions for both glucocorticoid versus salbutamol (45 participants) and glucocorticoid and salbutamol versus epinephrine comparisons (30 participants), with no differences between arms (Analysis 5.1; Analysis 7.1). There were no admissions in another trial including the latter comparison, as well as glucocorticoid and epinephrine versus salbutamol (Analysis 6.1; Analysis 7.1). Barlas 1998 multi-arm trial also performed an unblinded comparison between systemic prednisolone and inhaled budesonide, with no statistically significant differences in admissions by day 1 (Analysis 8.1).

Secondary outcomes

When compared to glucocorticoid at 60 minutes, epinephrine use was associated with lower clinical scores (SMD 0.31; 95% CI 0.12 to 0.50) and higher oxygen saturation (MD -0.99; 95% CI - 1.46 to -0.52; units: %) (two trials, 442 participants), while heart rate was lower with glucocorticoids (MD -7.56 bpm; 95% CI - 11.34 to -3.79) and there were no differences in respiratory rate (Analysis 4.2; Analysis 4.3; Analysis 4.4; Analysis 4.5). There were no differences in the single trial assessing clinical scores and heart rate at later time points.

Salbutamol was also favored when compared to glucocorticoids, in clinical scores at 60 minutes and three to six hours (SMD 0.65; 95% CI 0.01 to 1.28; and SMD 0.70; 95% CI 0.06 to 1.34, respectively), but not at 120 minutes (Analysis 5.2). Heart rate at 120 minutes was lower in glucocorticoid group (MD -7.53 bpm; 95% CI -14.28 to -0.78) and there were no differences in oxygen saturation at any time point (Analysis 5.3; Analysis 5.4).

At 3 to 10 days, clinical scores and respiratory rate results favored glucocorticoids and epinephrine as compared to salbutamol (SMD -1.22; 95% CI -1.98 to -0.46,

Table 2.7: Hospital re-admissions and return healthcare visits (in- and outpatients)*

Study	Population	Duration of follow-up	Glucocorticoid-including group	Placebo or comparator group	Notes
GLUCOCORTICOID AND EPINEPHRINE versus PLACEBO: HOSPITAL RE-ADMISSIONS					
Roosevelt 1996	Inpatients	Days 1 to 14	0	0	(No events in either group)
Klassen 1997	Inpatients	Days 1 to 7	4/35 (11%)	1/32 (3%)	P = 0.36
Teeratakulpisarn 2007	Inpatients	Days 1 to 30	3/89 (3%)	7/85 (8%)	
Roosevelt 1996	Inpatients	Days 1 to 14	0	0	(No events in either group)
GLUCOCORTICOID AND EPINEPHRINE versus PLACEBO: HOSPITAL RE-ADMISSIONS					
Plint 2009 (epinephrine - E; dexamethasone - D; placebo - P)	Outpatients	Days 1 to 22	D + E 95/199 (48%)	P + E 93/198 (47%)	Return to the health care provider for bronchiolitis symptoms; Difference between dexamethasone + placebo versus placebo + placebo, was significant in the unadjusted analysis (P = 0.04)
			D + P 106/199 (53%)	P + P 86/201 (43%)	
Schuh 2002	Outpatients	Days 7 to 28	9/35 (26%)	14/32 (44%)	Medical visits for continuing symptoms; P = 0.069
Klassen 1997	Inpatients	Days 1 to 7	29/35 (83%)	24/32 (75%)	P = 0.77
Roosevelt 1996	Inpatients	Days 1 to 14	16/65 (25%)	5/53 (9%)	P = 0.01; reported on visits made by the physician; 69% were for non-respiratory difficulties
Teeratakulpisarn 2007	Inpatients	Days 1 to 30	17/89 (19%)	26/85 (31%)	Visit to emergency room or a private clinic because of respiratory symptoms
GLUCOCORTICOID versus EPINEPHRINE: RETURN HEALTHCARE VISITS					
Plint 2009 (dexamethasone + placebo versus epinephrine + placebo)	Outpatients	Days 1 to 22	106/199 (53%)	93/198 (47%)	-
GLUCOCORTICOID AND EPINEPHRINE versus PLACEBO: RETURN HEALTHCARE VISITS					
Plint 2009 (dexamethasone + epinephrine versus placebo + placebo)	Outpatients	Days 1 to 22	95/198 (48%)	86/201 (43%)	-

*Berger 1998: no difference between groups, but did not report quantitative data. Data presented as n/N (%)

Table 2.8: Symptoms and quality of life (in- and outpatients)*

Study	Population	Duration of follow-up	Parameter	Glucocorticoid-including group	Placebo or comparator group	Notes
GLUCOCORTICOID versus PLACEBO						
Teeratakulpisarn 2007	Inpatients	Days 1 to 30	Time from treatment to being symptom free - mean ± SD	7.0 ± 5.9	9.0 ± 6.4	P = 0.035
Cade 2000	Inpatients	Days 1 to 28	Time taken for half of infants to become asymptomatic for 48 hours (95% CI) - time to event analysis	10 (10 to 13)	12 (10 to 16)	HR 1.41 (95% CI 0.98 to 2.04), P = 0.07
			Days with coughing or wheezing episodes - mean ± SD	17.0 ± 7.6 days	17.1 ± 8.5	Mean difference: 0.91 days (95% CI -2.72 to 2.41), P = 0.91
Roosevelt 1996#	Inpatients	Day 10 to 14	No current difficulty breathing - n/N (%)	45/45 (100)	37/42 (88)	P = 0.07
			Feeding and drinking well - n/N (%)	45/45 (100)	40/42 (95)	P = 0.57
GLUCOCORTICOID versus PLACEBO, GLUCOCORTICOID versus EPINEPHRINE, GLUCOCORTICOID AND EPINEPHRINE versus PLACEBO						
Plint 2009 (epinephrine - E; dexamethasone - D; placebo - P)	Outpatients	Days 1 to 22	Time to return to normal feeding - median (IQR)	D + E: 0.6 (0.2 to 1.3) D + P: 0.8 (0.3 to 1.9) P + E: 0.5 (0.2 to 1.2) P + P: 0.9 (0.3 to 2.1)		Time to return to normal feeding - mean symptom duration ratio D + E versus P + P: 0.63 (unadjusted 95% CI 0.5 to 0.8)¶; Time to return to quiet breathing - mean symptom duration ratio D + E versus P + P: 0.83 (unadjusted 95% CI 0.69 to 1.00); No other reported comparison was statistically significant in adjusted analysis
			Time to return to normal sleeping - median (IQR)	D + E: 0.7 (0.2 to 1.7) D + P: 0.8 (0.3 to 1.9) P + E: 0.8 (0.3 to 1.9) P + P: 0.8 (0.3 to 1.8)		
			Time to no coughing - median (IQR)	D + E: 12.6 (7.8 to 18.5) D + P: 13.8 (8.5 to 20.2) P + E: 13.2 (8.1 to 19.3) P + P: 13.3 (8.2 to 19.5)		
			Time to quiet breathing - median (IQR)	D + E: 3.1 (1.4 to 6.1) D + P: 3.7 (1.6 to 7.1) P + E: 3.6 (1.5 to 6.9) P + P: 3.7 (1.6 to 7.2)		

*Units in days unless otherwise stated; no study assessed or reported data from generic or disease-specific quality of life instruments; Richter 1998 also reported number of symptom-free days for a 6-week follow-up period

#Roosevelt 1996 primary outcome was time to resolution (defined as number of 12 h periods needed to achieve: O2 saturation > 95% at room air, accessory muscle score = 0, wheeze = 0 or 1, and normal feeding); only association measures were reported: HR 1.3 (95% CI 0.9 to 1.3), P = 0.22

¶time to symptom relief was analysed by means of parametric survival models with Weibull distributions assumed; 95% CI adjusted for multiple analysis in a factorial trial.

CI = confidence interval, HR = hazard ratio, IQR = interquartile range, SD = standard deviation

Table 2.9: Harms - adverse events						
Adverse event		**Nr of participants**	**Study**	**Glucocorticoid-including group, N (%)**	**Placebo or comparator group, N (%)**	**Notes**
GLUCOCORTICOID versus PLACEBO, GLUCOCORTICOID versus EPINEPHRINE, GLUCOCORTICOID AND EPINEPHRINE versus PLACEBO						
Gastro-intestinal	Vomiting	1466	Plint 2009*	D + E: 2/199 (1) D + P: 5/199 (2.5)	P + E: 4/198 (2) P + P: 3/201 (1.5)	Observed in the emergency department by research nurse
			Kuyucu 2004*	No events in either group (D + E, D + S, P + E, P + S)		Methods/timings NR
			Corneli 2007	16/305 (5.2)	14/295 (4.7)	Within 20 minutes after administration of the study medication
	Bleeding	1576	Corneli 2007	No events in either group		#
			Teeratakul pisarn 2007	2/90 (2)	1/89 (1)	Occult blood; also assessed diarrhoea separately. Methods/timings NR
			Plint 2009	D + E: 17/199 (8.5) D + P: 12/199 (6)	P + E: 14/198 (7) P+P: 16/201 (8)	Dark stools; reported by families during the 22-day telephone follow-up. No patient had more than 1 episode
Endocrine	Hypertension	1397	Plint 2009	D + E: 0/199 (0) D + P: 1/199 (0.5)	P + E: 1/198 (0.5) P + P: 0/201 (0)	Observed in infants admitted to hospital
			Corneli 2007	No events in either group		#
Infectious	Pneumonia	851	Corneli 2007	1/305 (3.3)	2/295 (7)	Also assessed empyema separately
			Teeratakul pisarn 2007	0/90 (0)	3/89 (3.4)	Methods/timings NR
			Klassen 1997	1/35 (3)	1/37 (3)	Methods/timings NR
	Varicella	1397	Corneli 2007	No events in either group		#
			Plint 2009	No events in either group		Reported by families during the 22-day telephone follow-up
General	Tremor	866	Kuyucu 2004	No events in either group		Methods/timings NR
			Plint 2009	D + E: 4/199 (2) D + P: 5/199 (2.5)	P + E: 4/198 (2) P + P: 2/201 (1)	Observed in the emergency department by research nurse
	Pallor/ flushing	866	Kuyucu 2004	No events in either group		Methods/timings NR
			Plint 2009	D + E: 23/199 (11.5) D + P: 15/199 (7.5)	P + E: 22/198 (11.1) P + P: 16/201 (8)	Observed in the emergency department by research nurse

Table 2.9: Harms - adverse events	
Additional reported adverse events: Goebel 2000 reported toxicity data: one patient was "jittery"; no evidence of further treatment complications. Plint 2009 also reported hyperkalaemia observed in infants admitted to hospital (only one case was noted in the dexamethasone group). *epinephrine - E; dexamethasone - D; salbutamol - S; placebo - P #Corneli 2007: Study clinicians and research assistants monitored the infants for adverse events during observation in the emergency department. Subsequent adverse events were determined at follow-up. A patient safety committee, made up of people not involved with patient enrolment, tracked all adverse events.	

and MD -13.70; 95% CI -20.56 to - 6.84, respectively) (Analysis 6.2; Analysis 6.3; Analysis 6.4). There were no other differences at earlier time points and regarding heart rate. Oxygen saturation at 60 and 120 minutes was higher in the epinephrine group when compared to glucocorticoid and salbutamol (MD -1.54; 95% CI -2.85 to -0.23, and MD -1.27; 95% CI-2.41 to -0.13, respectively) (Analysis 7.2; Analysis 7.3; Analysis 7.4; Analysis 7.5). No other statistically significant differences were found in clinical scores, oxygen saturation or respiratory or heart rate at other time points.

When comparing systemic prednisolone and inhaled budesonide, oxygen saturation results favored budesonide at 60 minutes and 120 minutes (MD -1.46; 95% CI -2.74 to -0.18, and MD -1.73; 95% CI -3.06 to -0.40, respectively), and heart rate was lower with prednisolone at three to six hours (Analysis 8.3; Analysis 8.4). No differences were found in all other outcomes and time points (Analysis 8.2).

Plint 2009 reported safety assessments comparing glucocorticoid and epinephrine (Table 2.9). Pallor was observed in 7.5% of participants in the glucocorticoid group, compared to 11.1% in the epinephrine group. There were no significant differences in vomiting, bleeding, hypertension, varicella and tremor between glucocorticoids and epinephrine. No other trial from any of the other comparisons reported adverse events data.

DISCUSSION

Summary of main results

Results from this review do not suggest a clinically relevant stand-alone effect of systemic or inhaled glucocorticoids in either outpatient and inpatient settings (Summary of findings for the main comparison). There were no statistically significant differences in outpatient admissions by days 1 and 7, and pooled RR

estimates favoring glucocorticoids versus placebo were below commonly used thresholds for clinical relevance. Strength of evidence was moderate to high, indicating our confidence in these effect estimates. There were also no differences in secondary outcomes, particularly clinical scores, oxygen saturation and respiratory symptoms. For inpatient trials, precise and consistent results did not show differences in LOS as compared to placebo. The lower boundary of the pooled estimate confidence interval was about nine hours, likely excluding a clinically relevant benefit from glucocorticoids. While clinical score results were superior during the first day of treatment, no consistent differences were found at later time points or in any other secondary outcomes. Subgroup analyses according to age and RSV status did not suggest effect modification by these factors; heterogeneity did not allow adequate analysis of atopy and glucocorticoid type or dose.

Exploratory evidence suggests that combined glucocorticoids and bronchodilators may have clinically relevant benefits. A large factorial trial with low risk of bias found that high-dose dexamethasone with epinephrine reduced admissions by day 7 when compared to placebo, in outpatients with moderately severe bronchiolitis (Summary of findings 2). The unadjusted RR reduction estimate was 36%, and 11 children with bronchiolitis had to be treated to reduce one admission given the study's baseline risk. Clinical scores and symptoms results supported this benefit. However, these are the findings of a single study and should be interpreted cautiously. There were methodological issues with trial design and results may have arisen by chance. Further evidence regarding combined therapy is scarce and imprecise, and exploratory subgroup analysis was not conclusive as to an additive/synergistic effect of glucocorticoids combined with bronchodilators.

No relevant differences were found in short-term general and intervention-specific adverse effects for these comparisons. However, balancing harms and benefits of glucocorticoids alone or combined was hampered by the lack of long-term safety data.

Overall completeness and applicability of evidence

The heterogeneous definition of bronchiolitis is often a motive for controversy when interpreting trial and review results.[588,590,784] There is no international consensus

due to variation in semantics and clinical findings (for example, in the UK, 'crackles' are often key to diagnosis, as opposed to 'wheeze' in North America).[4] A first episode of wheezing may be a manifestation of wheezing phenotypes with heterogeneous biological, genetic, viral or environmental determinants, and distinct prognosis.[7,458,460] However, research is still ongoing to identify simple, valid and universal discriminative and prognostic tools to prospectively distinguish between them.[460,785,786] We used a pragmatic definition and focused on first time wheezing so results could be directly pertinent to infants with 'typical' viral bronchiolitis, as opposed to those with acute recurrent wheezing or asthma.

We found variability in both bronchiolitis severity and glucocorticoids schemes, but this did not affect the consistency of results. Baseline disease in outpatients was often moderate, but the use of different clinical criteria and scales limited the comparison be- tween trials, particularly for inpatients. The wide range of control group admission rates and LOS can be partially explained by differing disease severity, but it also reflects variation in bronchiolitis management, for example, different admission/discharge criteria and standards of care.[9,44,81,395,549,553,557] Our findings were consistent in trials performed worldwide, and results likely apply to settings with different resources and management strategies.

Most studies were restricted to healthy infants, often excluding children with chronic conditions and prematurity. Lack of evidence for this subset of patients is problematic, since many are particularly at risk of adverse outcomes.[193,283,351] Epidemiological studies have highlighted the short- and long-term impact of RSV disease in prematurity, and underlying changes in respiratory pathophysiology may limit the external validity of our results in these populations.[283]

Results from subgroup analyses did not identify any subset of participants with a different response to glucocorticoids. Older aged and atopic children are at higher risk of recurrent wheezing and asthma, and both factors have been traditionally proposed as markers of underlying glucocorticoid-responsive phenotypes in first-time wheezers.[590,787] We found no conclusive evidence of such effect with age. We were unable to study atopy, but subgroup analyses from individual studies did not identify any significant differences. Specific viruses may also modulate response, as RSV and rhinovirus infections are associated with recurrent wheezing and the latter

is a stronger predictor and possibly more responsive to glucocorticoids.[512646,788,789] We found no differences according to RSV status, while other viral etiologies were not reported. Accumulating evidence shows that glucocorticoids have reduced effectiveness in later acute recurrent wheezing.[647,648] Further, each of these factors per se has limited prognostic accuracy in defining stable wheezing phenotypes.[460,776,790] Our results suggest that 'typical' viral bronchiolitis is not glucocorticoid-responsive. Potential methodological limitations include the use of aggregated data and heterogeneity in definition, ascertainment and reporting of subgroups.

We found promising exploratory results from one large trial using combined dexamethasone with epinephrine for moderately ill outpatients. Although reliance on findings from single precise well-conducted trials is often reasonable, in this factorial trial the additive interaction between treatments was unanticipated, and this limits the interpretation of its results.[658,758] Our observational and exploratory subgroup analyses of protocolized bronchodilators may indirectly support an additive effect, but findings were not conclusive for both outpatients and inpatients. The latter are often a separate population due to differences in severity, duration of symptoms or non-response to initial bronchodilators, and these may affect response to therapy. Replication is therefore needed to improve our confidence in the direction, precision and magnitude of the effect estimates for outpatients, and its applicability for inpatients. Whether results from combination therapy can be generalizable to different glucocorticoid or bronchodilator schemes is also not known. Systemic dexamethasone is favored in another common viral respiratory disorder, croup.[791] Its long half-life and stronger potency may account for its effect, but underlying pathological changes are distinct between these two conditions. Plint 2009 used multiple high doses of dexamethasone. A previous dose-finding trial suggested similar results with a single high dose, although there was no placebo comparator; the lowest efficacious dose remains unknown.[792] The choice of bronchodilator is also undecided. A recently updated Cochrane review on epinephrine in bronchiolitis showed a reduction in first day outpatient admissions, as well as other short-term severity outcomes.[775,793] This might explain part of the early benefit of combined therapy seen in Plint 2009. Further research is needed to clarify whether combined epinephrine is superior to combined salbutamol, particularly given the variation in bronchodilator choice in practice.

Evidence from basic and translational research may support a synergistic effect of combined therapy, but it is not clear how this reconciles with the limited effect of glucocorticoids alone. Inflammation pathways and mediators involved in bronchiolitis seem to be distinct from those in glucocorticoid-sensitive asthma. Innate immunity, specific cytokine dysregulation patterns and neutrophilic inflammation may be relevant for some early wheezing phenotypes, which could explain the limited biological action of glucocorticoids alone.[643,646,783,784] Paradoxically, clinical and biological synergism between glucocorticoids and bronchodilators has been a major topic in asthma treatment.[763] Two-way molecular interactions exist, including β2-agonist-stimulated glucocorticoid-mediated gene transcription and glucocorticoid-induced increase in the transcription of the ß2-receptor gene.[650] Epinephrine's α-adrenergic vasoconstricting and edema-reducing activity could confer an additional short-term benefit. Whether these mechanisms are involved in acute bronchiolitis therapy, and the role of specific types and doses of bronchodilators and glucocorticoids, is unknown.

These positive results should be balanced against incomplete data on harms. Safety concerns are expected when considering the widespread use of epinephrine and glucocorticoids in young children with viral wheezing, particularly with repeated high glucocorticoid doses.[647,764] Current data from RCTs and observational studies in croup suggest a favorable short-term safety profile from both dexamethasone and epinephrine.[601,791] Considering all trials, our results do not suggest any serious or frequent short-term expected or unexpected harms from glucocorticoids in the absence of co-morbidities. However, the power to detect important differences was limited due to the infrequent occurrence of events, and adverse event detection was heterogeneous. Glucocorticoids also raises long-term safety issues. Their use in prematurity for neonatal respiratory distress has been associated with effects on adrenal function, cardiovascular responses, somatic and lung growth, and neurodevelopment.[765-768] Evidence is scarce, however, regarding effects of short-term use in otherwise healthy term infants, and none of these were studied in included trials. Further pharmacoepidemiologic data are needed to permit adequate short and long-term risk-benefit assessments.

Quality of the evidence

Two key factors affected the strength of evidence: potential risk of bias in the included studies, and sparsity of data for many of the outcomes and comparisons, with imprecise estimates and unknown consistency across studies.

A majority of trials had unclear risk of bias, usually due to in- complete or inadequate reporting, and many comparisons only included small trials at high risk of bias. Inadequate allocation concealment and blinding were likely to be relevant given the nature of interventions (for example, inhaled versus systemic administration) and outcome assessments (for example, physician-based admissions or discharge decisions). Incomplete outcome data were often found, with losses of follow-up in outpatient trials. However, for the main glucocorticoid versus placebo comparison, sensitivity analyses restricted to low risk of bias trials did not change the direction or magnitude of results for primary outcomes, highlighting their consistency.

Sparsity of data was a result of a large number of comparisons as well as variability in the choice of outcomes and timing of assessments. Within trials, this also led to frequent uncertainties regarding selective outcome reporting. The message around consistency and relevance of outcomes is not new to this field.[589,693,770] The absence of standardized, validated and patient-important outcome measures has been a serious threat to bronchiolitis trial validity. Our primary outcomes focused on hospital use, which has clear implications for patients, families and health services. However, there is no guidance supporting the choice of methodologically sound and patient-important outcomes. Lack of reporting of admission and discharge criteria is also problematic given the wide variation in bronchiolitis management. Additionally, the choice of clinical scales was inconsistent. RDAI was used in a considerable number of trials, but its clinimetric properties - for example, responsiveness and interpretability - are not well known, which limits the interpretation of findings. This was compounded by the absence of quality of life measures. Further work is needed to define a core set of clinically important efficacy and safety outcome measures and timing of assessments, for trials and systematic reviews in this field.

Potential biases in the review process

Some limitations have already been described, others should also be highlighted. We did not obtain further data from authors of included studies, which might have clarified 'Risk of bias' assessments and further added to reported trial characteristics and secondary outcome results. There is scarce guidance on how to investigate synergism/antagonism at a systematic review level, therefore our approach should be considered exploratory, including our use of factorial trial results. However, we performed sensitivity analyses of different analysis methods and these did not show a change in the direction of results. Our choice of outcome time intervals may have been a source of heterogeneity, although it was limited by the sparsity of reported data. Limitations of subgroup analyses are well known and have been addressed. Grading of evidence was limited by the lack of guidance regarding clinically relevant differences in studied outcomes.

Agreements and disagreements with other studies or reviews

Two previous non-Cochrane systematic reviews assessed the use of glucocorticoids in acute bronchiolitis, one of which also performed meta-analysis.[587,589] None of the reviews included data from the two recent large glucocorticoid outpatient trials. There was some discordance in inclusion criteria regarding population and interventions: Garrison 2000 only included inpatient trials and was restricted to systemic glucocorticoids, and no review excluded previous wheezing. The choice of primary outcomes and their definitions, timings and analysis also differed. While Garrison 2000 highlighted a statistically significant reduction in LOS for inpatients, this analysis used a modified outcome definition. When comparing similar analyses for this outcome, quantitative results were comparable between all reviews, including ours, and suggest no relevant benefit from glucocorticoids in inpatients. Outpatient descriptive and quantitative results from King 2004 also found no difference in admissions. No previous review assessed the hypothesis of synergism between glucocorticoids and bronchodilators at an analysis level, while subgroup analyses assessing possible dose-response and effect modifiers like age and RSV status showed similar negative results.

AUTHORS' CONCLUSIONS

Implications for practice

Current evidence does not support a clinically relevant effect of systemic or inhaled glucocorticoids on admissions or length of stay, when used alone in infants with

bronchiolitis defined as a first episode of wheezing. Clinical score results suggest some short-term benefit of glucocorticoids for inpatients, but no differences were found in other secondary outcomes. Absence of treatment effects was consistent throughout studies despite substantial heterogeneity regarding included populations, interventions and outcomes, and this finding is likely to be applicable in diverse settings.

Exploratory results from a single large trial suggest combined high-dose systemic dexamethasone and epinephrine may reduce outpatient admissions in moderately severe bronchiolitis. These findings should be interpreted cautiously and may have arisen by chance. While no relevant differences were reported in short-term adverse events, long-term safety data were missing. Efficacy, harms and applicability of combined therapy need to be clarified further.

Implications for research

A large randomized controlled trial is needed to replicate and complement findings from combination therapy with glucocorticoid and bronchodilator for outpatients. Additional aims could include assessing the minimum efficacious glucocorticoid dose and the most adequate co-intervention. This strategy could also be tested in inpatient settings. Choice of comparators should take into account the wide variability in bronchodilator use, so that valid results may be more easily implemented. Further investigation of parent-reported outcomes is needed, as well as data to assess the long-term safety of this association. Future trials should use standardized sets of outcome measures in this field.

CHAPTER 3

OUTCOMES AND MEASUREMENT INSTRUMENTS IN BRONCHIOLITIS TRIALS

3.1 Exploratory Review Of Outcome Domains And Measurement Instruments In Trials Of Bronchiolitis

3.2 Measurement Properties Of The RDAI and RDAI Scales In Bronchiolitis

3.1 EXPLORATORY REVIEW OF OUTCOME DOMAINS AND MEASUREMENT INSTRUMENTS IN TRIALS OF BRONCHIOLITIS

Presented at:
Fernandes R. Bronchiolitis core outcome set. Third meeting of the COMET (Core Outcome Measures in Effectiveness Trials) Initiative, Liverpool, 20 June 2013.

Background

Selection of appropriate primary and secondary outcomes is essential for study design, as ultimately, any study is only as credible as its endpoints.[668] To be useful, clinical trials that evaluate benefits and harms of interventions must select outcomes of relevance to stakeholders, and measure them using instruments with adequate measurement properties.[669]

As discussed in Chapter 1, inconsistent selection, measurement, and reporting of outcomes in clinical trials raises three main problems.[673,680,699] First, outcomes may not consistently reflect endpoints that are meaningful for all stakeholders, particularly parents and caregivers, or physicians in different settings. Second, inconsistency in measurement domains and instruments is a barrier to compare, contrast, and combine trial findings, which will inevitably affect their interpretation and future uptake. Third, if researchers have measured a particular outcome in a variety of ways, outcome reporting bias may ensue.

These issues could be addressed with the development and application of agreed standardized sets of outcomes, i.e. 'core outcome sets', that are important to relevant stakeholders, e.g. patients and health care practitioners.[672,673,681] Methods to support the development and implementation of core outcome sets are still evolving.[682,684,691] One must distinguish between potential domains (''what to measure'') and measurement instruments (''how to measure''); and the process to identify these and to reach consensus on which to include in a core set.[691] Stepwise approaches for core outcome set development have been proposed, all of which suggest literature review as a starting point.

One of the key limitations identified by most systematic reviews of treatments in bronchiolitis has been the heterogeneity in the selection of outcomes and measurements instruments reported in clinical trials (Chapter 2).[695] However, the extent of these gaps in outcome measurement is not known. A review of previous trials and/or systematic reviews can provide evidence on which outcome domains have been measured, which measurement instruments have been used and timings of measurement. Gaps and discrepancies identified help inform core outcome set development and consensus procedures with stakeholders.[682,691]

Based on a recent overview of reviews of treatments for bronchiolitis, we designed a preliminary study to document domains and instruments used to date in bronchiolitis.[10]

Objectives

Our primary objective was to identify outcome domains and measurement instruments reported in clinical trials of bronchiolitis included in previous Cochrane systematic reviews.

Our secondary objectives included:

- to stratify outcome domains by setting in which the trial was conducted;
- to match measured outcome domains to existing conceptual frameworks, and identify gaps in measured domains;
- to identify timings of measurement;
- to identify outcomes used for sample size calculation ("primary" outcomes);
- to identify primary outcomes of each systematic review.

Methods

Criteria for considering trials for inclusion

We included RCTs examining pharmacologic or non-pharmacologic interventions for the treatment of bronchiolitis in children, provided they were included in one of the 11 Cochrane reviews of bronchiolitis treatments of a previously published overview of reviews on treatment of bronchiolitis.[10] Inclusion criteria for these trials regarding population, interventions and outcomes, was dependent on the decisions and methods used within each systematic review.

Search methods for identification of trials

We identified the references of all trials included in each of the 11 Cochrane reviews included in an overview of reviews on treatment of bronchiolitis.

Data collection and analysis

One reviewer (RF) extracted the following information using a standardized data collection form:

- from each systematic review: type of intervention, number of trials, sample sizes, trial setting and primary outcomes
- from each trial: setting in which the trial was conducted (outpatient, inpatient, intensive care), all reported outcomes, corresponding measurement instruments when applicable, timings of measurement, and outcomes on which sample size calculations were based.

We analyzed outcomes, instruments and timings of measurement by setting.

Further, we classified reported outcomes into domains and subdomains using two conceptual frameworks: one adapted from a scheme proposed by Sinha et al, and another adapted from a recently proposed conceptual frameworks of core areas, outcome domains and subdomains by the OMERACT group (Filter 2.0).[691,794] The adapted frameworks with examples applied to bronchiolitis are shown in Table 3.1.

Table 3.1: Classification of outcomes, outcomes domains and subdomains according to conceptual frameworks

Adapted from Sinha et al		Adapted from Boers et al		Bronchiolitis (sub)domains		Outcomes/ instruments - examples reported in included trials	Notes
Domain	**Subdomain**	**Core Area**	**Domain**				
Disease activity	Clinical severity	Pathophysiological manifestations	Reversible manifestations/organ function	Clinical severity	Respiratory distress	Physical examination findings (e.g. respiratory rate, retractions, auscultatory findings); measures of oxygenation/ ventilation (e.g. oxygen saturation, duration of oxygen therapy); use of respiratory rescue therapy (e.g. bronchodilators); respiratory distress instruments	Mostly relates to short-term symptoms; measures can be "direct" (e.g oxygen saturation) or "indirect" (e.g . duration of oxygen therapy); most are clinician-based (measurement by clinician or implies clinical decision-making); variable metrics (e.g. continuous, using threshold cut-offs, duration or time-to-resolution); timing varies
					Other dimensions of disease severity	Hydration status, feeding tolerance, general well-being	
					Global measures of disease severity	Clinical severity instruments (including measures from more than one subdomain)	
					Symptoms - caregiver-reported	Respiratory and/or other dimensions (e.g. cough, wheezing, feeding)	Mostly relates to short-term symptoms; most are caregiver-reported; same variation in parameters as above
	Radiological		Organ function	Radiological		Chest radiography findings (e.g. using radiograph assessment score)	

Table 3.1: Classification of outcomes, outcomes domains and subdomains according to conceptual frameworks

Adapted from Sinha et al		Adapted from Boers et al		Bronchiolitis (sub)domains		Outcomes/ instruments - examples reported in included trials	Notes
Domain	**Subdomain**	**Core Area**	**Domain**				
	Lung function tests			Lung function tests		Pulmonary mechanics (e.g. total pulmonary resistance, dynamic compliance)	
	Biomarkers		Biomarkers	Other		Serum or bronchoalveolar lavage markers of inflammation (e.g. cytokines)	
Physical consequence of disease	Disease progression		Irreversible manifestations	Recurrent wheezing and asthma		Wheezing exacerbations, wheezing symptoms, medication for wheezing, respiratory questionnaires	Relates to long-term symptoms; may overlap with caregiver-reported symptoms
	Mortality	Death		Mortality		Mortality	
Functional status		Life impact					
Quality of life/family outcomes			Quality of life	Quality of life		Child and/or caregiver quality of life; caregiver functional status	
			Secondary impact on family caregivers				
Health resource use		Resource use/ economical impact	Health care	Health resource use	Hospital-related	ED visits, hospital admissions, length of stay in hospital or intensive care unit	
					Community-related	Outpatient visits (scheduled/ unscheduled)	
Adverse effects of therapy	Short-term Long-term	Adverse effects across core areas and domains		Adverse effects		Adverse events (general or intervention-specific)	

Results

We included 90 unique studies from the 11 systematic reviews. Table 3.2 presents characteristics of each included systematic review, including setting, intervention and primary outcomes. Nine trials were conducted in the intensive care, 53 with inpatients, 19 in the ED and 9 in ambulatory settings; two trials were conducted both in the ED and outpatient clinics.

Only three reviews selected all same primary outcomes (i.e. rate of admission and length of stay for inpatients); two of these reviews were conducted by the same group. Clinical severity and respiratory distress were the most frequently chosen outcome domain and subdomain (7/11 reviews), followed by healthcare resource use (5/11).

Figure 3.1 presents the distribution of reported outcomes in included trials, by relevant domains/subdomains of bronchiolitis. The reported domains or subdomains were, by descending order of frequency: clinical severity (i.e. respiratory distress and other dimensions of disease severity, clinician-reported) (97%), healthcare resource use (59%), adverse effects (28%), non-clinics markers of disease activity and organ function (e.g. lung function tests, radiological findings) (16%), caregiver-reported symptoms (14%), mortality (8%), recurrent wheezing and asthma (6%); only two studies (1%) assessed quality of life outcomes.

Only 38 trials (42%) explicitly reported which outcomes were used for sample size calculation. The domains of these outcomes were, by descending order of frequency: clinical severity (55%), healthcare resource use (29%), recurrent wheezing and asthma (8%), caregiver-reported symptoms (5%), and non-clinical markers of disease activity and organ function (3%) (Figure 3.1).

Table 3.2: Characteristics of included systematic reviews

Review title, authors	Number of studies, sample size (range)	Trial setting	Intervention	Primary outcomes	
				Defined	Corresponding bronchiolitis (sub)domains
Antibiotics for bronchiolitis in children	1, 52	Inpatient	Antibiotics (oral, intravenous, intramuscular or inhaled)	Time for the resolution of symptoms/signs: pulmonary markers; respiratory distress; wheeze; crepitations; oxygen saturation; and fever.	Clinical severity, respiratory distress
Bronchodilators for bronchiolitis	29, 1912 (16–186)	Inpatient, outpatient and intensive care unit	Bronchodilator therapy (nebulized, oral or subcutaneous)	Oxygen saturation	Clinical severity, respiratory distress
Chest physiotherapy for acute bronchiolitis in paediatric patients between 0 and 24 months old	3, 172 (32–90)	Inpatient	Any type of chest physiotherapy	Change in the severity status of bronchiolitis; oxygen saturation levels; transcutaneous PCO2	Clinical severity, respiratory distress
Epinephrine for bronchiolitis	17, 2010 (27–800)	Inpatient and outpatient	Epinephrine	Rate of admission by Day 1 and Day 7 for outpatients; length of stay for inpatients.	Healthcare use
Continuous negative extrathoracic pressure or continuous positive airway pressure for acute hypoxemic respiratory failure in children	1, 33	Intensive care unit	CNEP with or without assisted PPV or Ni-CPAP without assisted PPV	Mortality (both early and late, defined as less than and greater than 30 days after the diagnosis); improvement in oxygenation (at 24 hourly intervals up to one week) as measured by oxygenation index [mean airway pressure x fractional concentration of oxygen x 100)/ arterial oxygen tension (PaO2)], and hypoxia score [arterial oxygen tension/fractional inspired oxygen concentration ratio (PaO2/FiO2)] or improvement in PaO2 with reduction of FiO2 after starting the therapy; failure (death or use of any additional form of assisted ventilation).	Mortality; clinical severity, respiratory distress
Glucocorticoids for acute viral bronchiolitis in infants and young children	17, 2596 (32–800)	Inpatient and outpatient	Short-term systemic or inhaled glucocorticoids with or without co-interventions	Rate of admission by Day 1 and Day 7 for outpatients; length of stay for inpatients.	Healthcare use

Table 3.2: Characteristics of included systematic reviews

Review title, authors	Number of studies, sample size (range)	Trial setting	Intervention	Primary outcomes	
				Defined	Corresponding bronchiolitis (sub)domains
Heliox inhalation therapy for bronchiolitis in infants	5, 97 (12–39)	Intensive care unit	Inhaled heliox	In-hospital mortality; need for mechanical ventilation; need for endotracheal intubation; length of paediatric intensive care unit (PICU) stay; adverse effects	Mortality; clinical severity, respiratory distress; healthcare use; adverse effects
Nebulized hypertonic saline solution for acute bronchiolitis in infants	7, 581 (44–186)	Inpatient and outpatient	Nebulized hypertonic saline solution alone or with bronchodilator	Length of hospital stay or time taken to be ready for discharge (inpatients); rate of hospitalisation (outpatients or emergency department patients)	Healthcare use
Immunoglobulin treatment for respiratory syncytial virus infection	4, 311 (35–107)	Intensive care unit	Immunoglobulin	Mortality; length of hospitalisation; length of ventilation; oxygen dependence	Mortality; healthcare use; clinical severity, respiratory distress
Inhaled corticosteroids during acute bronchiolitis in the prevention of post-bronchiolitic wheezing	5, 374 (40–161)	Inpatient	Inhaled corticosteroids	Incidence of physician-diagnosed wheezing episodes.	Recurrent wheezing/asthma
Oxygen therapy for lower respiratory tract infections in children between 3 months and 15 years of age	4, 418 (80–121)	Inpatient	Oxygen administration using a facemask, head box or hood, nasopharyngeal catheter, nasal catheter, nasal prongs or nasal cannula, or nasal CPAP	Clinical failure report (defined as the need for any additional intervention or change in treatment, no improvement in signs or SaO2 values or progression of other clinical signs associated with more severe hypoxaemia in children with acute lower respiratory tract infections - cyanosis, neurological impairment, inability to drink, or death) by a maximum of two hours after the intervention; combined outcome of improvement of respiratory signs achieved during the first 24 hours after oxygen therapy was initiated (subcostal in drawings and fast breathing) and/or improvement of oxygen saturation measured either by arterial blood gases (SaO2) or by oximeter (SpO2); efficacy of non-invasive oxygen delivery methods is defined as the ability to relieve hyperaemia (note: these outcomes were not listed explicitly as primary outcomes)	Clinical severity, respiratory distress

We found differences between measured outcome domains by setting (Figure 3.2): trials in outpatient settings measured more frequently caregiver-reported symptoms than in inpatient settings (none in intensive care unit and 19% in inpatient trials, vs. 30% in ED and 44% in outpatient trials), with the inverse being true for other markers of disease activity such as lung function outcomes (44% in intensive care unit trials, vs. 5% in ED trials).

Most reported instruments were used to measure dimensions of clinical severity. A majority were respiratory distress scales, while a few also encompassed other dimensions of disease severity (e.g. feeding, global status). These instruments were usually based on clinical assessment by a health care practitioner. Twenty-three such scales were reported, with more than one scale being seldom used. The most frequently used scales were: the Respiratory Distress Assessment Instrument and the Respiratory Assessment Change Score, two-related instruments, either in their original or adapted versions (22 trials); the scale by Wang et al, either in its original or adapted version (11 trials); and the scale by Tal et al (four trials). Twenty other scales were developed and reported in only one trial. Even when trials used the same instrument, there was variability in specific metrics (e.g. end value vs change from baseline) and methods of analysis and aggregation (e.g. for change from baseline, use of different cut-offs, or mean values).

Most trials focused on measurement of short-term outcomes (Figure 3.3). Outcomes were measured most frequently during the first two hours after interventions (51% of trials), and from 6 to 24 hours, and from one to three days (both 39%). Only 30% of trials measured outcomes from three to 10 days, 14% from 10 to 30 days, 6% from one to six months, and 4% after six months. For trials in hospitalized patients, most but not all measured outcomes during the duration of admission (56% in intensive care unit and 73% in ward inpatient trials). We found some variability in timings of outcome measurement between settings. Only trials conducted in the ED and trials with inpatients measured longer-term outcomes after 10 days.

Discussion

In this exploratory analysis of a sample of clinical trials in children with bronchiolitis, we found that reported outcome measurements were mostly restricted to short-term clinician-based clinical severity/respiratory distress and healthcare use

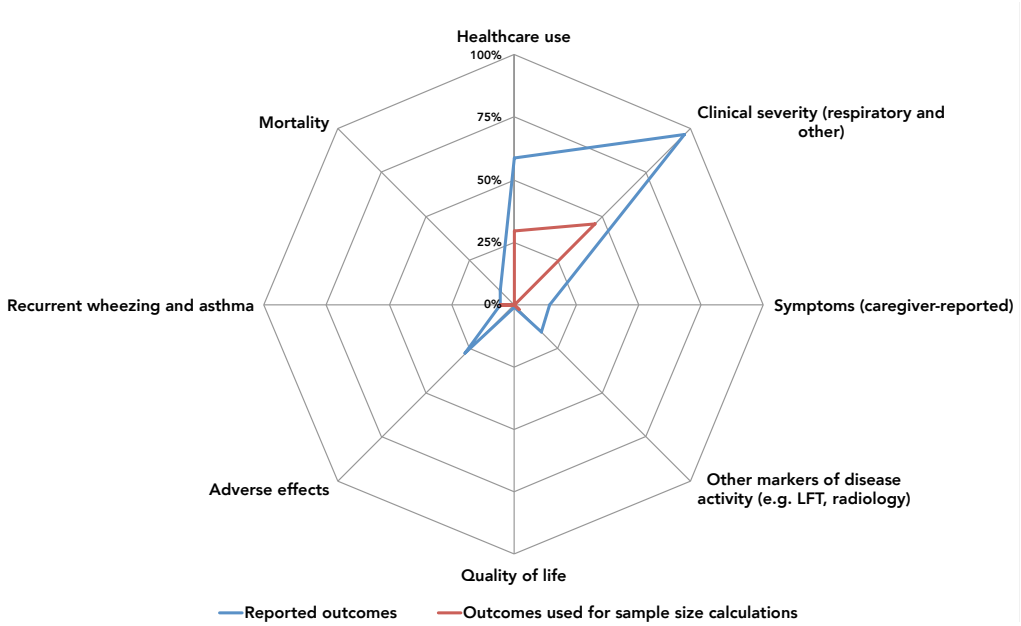


Figure 3.1. Spider chart of outcome domains reported and used for power calculations in bronchiolitis trials

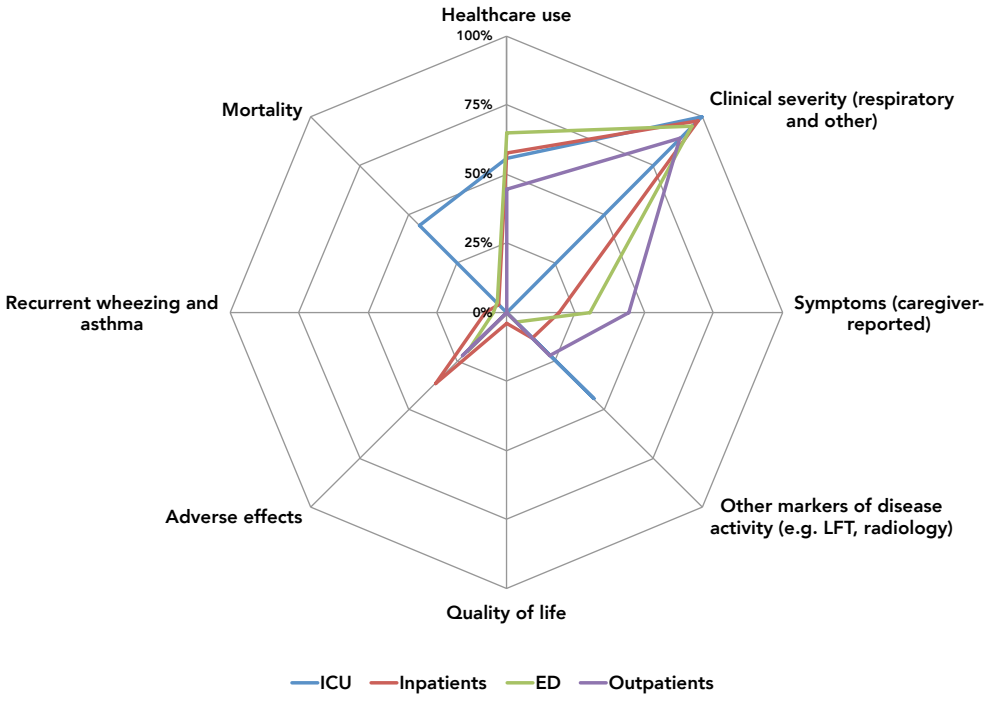


Figure 3.2. Spider chart of outcome domains reported in bronchiolitis trials by setting

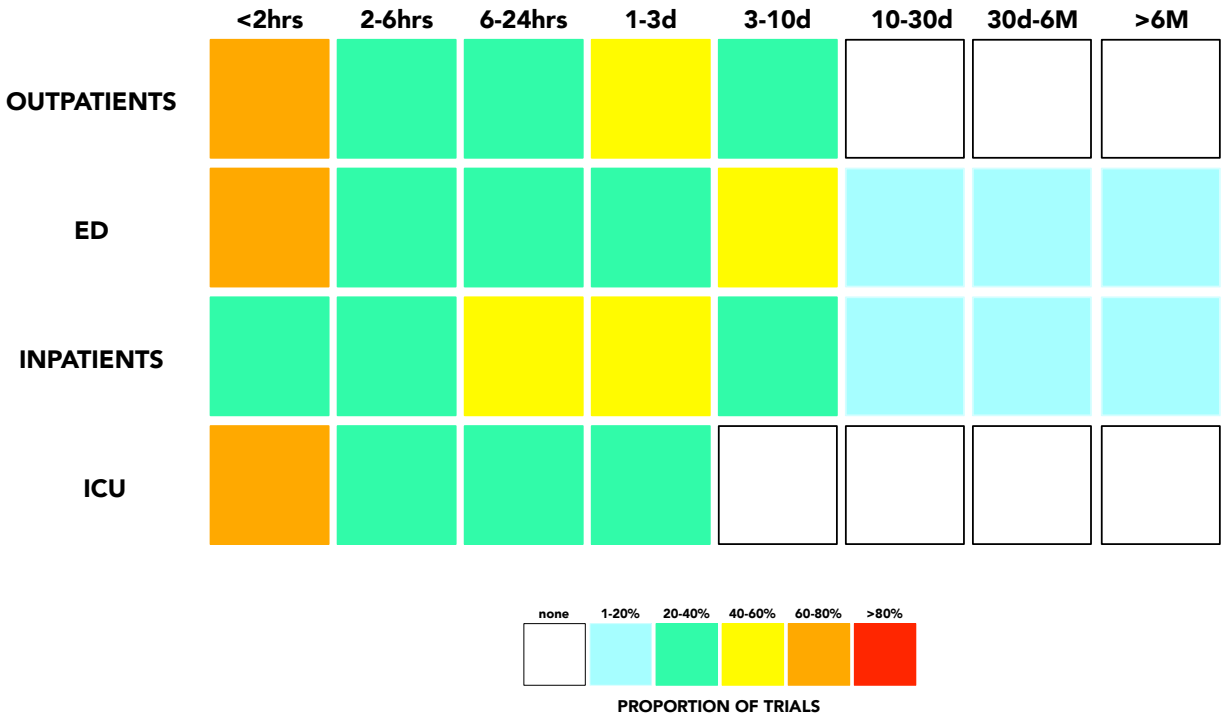


Figure 3.3. Matrix of timings of outcome measurement by setting

domains, while few measured caregiver-reported symptoms and quality of life, or long-term outcomes. The same was found for outcomes used to power these trials. Further, more than 20 different measurement instruments were identified, with different timings of measurement, metrics and methods of analysis. Systematic reviews used to identify our sample of trials also showed variability in their predefined primary outcomes. The absence of standardized, validated and patient-important outcome measures is a serious threat to the validity of bronchiolitis trial and systematic reviews. Further research should expand and confirm these preliminary results in order to support the development of a core set of patient-important outcomes and consistent measurement instruments with adequate measurement properties.

The focus on short-term outcomes related to clinical severity and respiratory distress is expected, given bronchiolitis is an acute respiratory condition. However, we found wide variability in specific outcome measurements and instruments used as well as their methods of analysis, leading to sparsity of data and limiting comparability of trial results based on these scales. Further, previous systematic

reviews have found that there is limited evidence regarding the measurement properties of many respiratory scales, i.e. their validity, reliability and responsiveness.[694] These instruments were often developed ad hoc, as shown by the 20 unique scales developed for individual trials in our sample; few if any have been specifically validated in children with bronchiolitis by current methodological standards. Thus there is scarce knowledge on whether they are suitable for use as outcome measures in clinical trials. Further, few studies have assessed the magnitude in change scores that are perceived as important, i.e. minimum important changes. This limits the interpretability of trial and systematic review results, and also the use of these scores for sample size calculations.

Health care resource use outcomes were measured by a considerable proportion of trials, often as primary outcomes. Most trials focused on measuring hospital admission rates and length of hospital stay. These are likely important measures for clinicians as well as health services, as bronchiolitis is a major cause of hospital admission, and inpatient health care costs are considerable. However, previous systematic reviews have found considerable variability in these outcomes, e.g. baseline risk of admission and length of stay, with few details on criteria for admission or discharge being reported. Many factors may influence hospital admission and discharge decisions beside individual factors related to clinical severity, including social issues, types of admission unit, health services constraints, local practices and protocols, and physician preferences. Given the wide practice variation in bronchiolitis, these factors may somewhat limit the use of outcomes such as hospital admission, length of stay and admission to intensive care as primary outcomes measures to infer a treatment effect. Further, other measures of resource use and economical impact, including ambulatory care and economical costs, were rarely measured.

Our results also show very few bronchiolitis trials measure caregiver-reported outcomes, either as measures of clinical severity (i.e. longer-term symptoms), or measures of life impact, including functional status and quality of life. This scarcity of parent or proxy-reported measures is problematic. First, it reflects that the patient perspective is hardly incorporated into current outcome measures. Previous studies in other conditions have found that life impact measures are outcomes of great importance to patients. Further, research has shown that bronchiolitis has a

substantial and negative impact in health-related quality of life, including functioning limitations in children and delayed return to normal family routine. Second, observational studies have found that a substantial proportion of children have protracted recovery and disease can last a few weeks, including respiratory and non-respiratory symptoms. Patient-reported instruments are needed to capture these symptoms during the recovery period, as these are of relevance to children for patients with bronchiolitis in all settings.

Most bronchiolitis trials focused on a very short time period of outcome measurement of a few days, and failed to assess long-term outcomes. Many trials of bronchiolitis are performed in acute settings, and interventions included in this sample of trials may not be expected to influence long-term outcomes. Further, limited trial resources are often a barrier to long-term follow-up in many pediatric trials. However, there is growing evidence linking bronchiolitis with recurrent wheezing and asthma, which are likely patient-important outcomes. Further, little insight can be gained from putative surrogate markers, since few trials assessed them, and there is no valid predictive marker for these later outcomes.

The following limitations should be considered. First, the range of patient population and interventions of included trials was limited, we relied on the search and screening of the Cochrane reviews, and we restricted our sample to RCTs. Some of the included Cochrane reviews have been recently updated. Further, we did not have access to trial protocols and did not assess possible selective outcome reporting, nor did we seek follow-up publications on these trials, e.g. to report long-term follow up. These factors may limit the external validity of our findings, i.e. other outcomes and relevant measurement instruments with longer timings of measurement may be reported in non-included bronchiolitis trials.

Second, our approach to the classification of outcome domains according to existing frameworks is exploratory. On the one hand, there is overlap between measures of some domains and subdomains, e.g. global scales of disease severity that encompass both respiratory distress and global status and feeding as other dimensions/subdomains of disease severity; measures such as duration of mechanical ventilation and oxygen therapy which include concepts of respiratory distress and also health resource use. We chose to distinguish clinician-based

measures from caregiver-reported ones even though they may measure the same (sub)domain, to highlight the lack of patient-reported outcomes. Further, timing of measurement is an important factor for (sub)domain classification, e.g. parent-reported symptoms may include shorter-term respiratory symptoms and longer-term recurrent wheezing. Also, criteria for classification of outcomes such as those pertaining to adverse effects is debatable, as some outcomes, e.g. clinical severity or hospital use, are possible adverse effects of many interventions. On the other hand, the conceptual frameworks themselves are emerging, and evidence is needed to support them.

This preliminary assessment of outcomes and measurement instruments reported in bronchiolitis trials highlights gaps in current trial design that seriously affect trial validity and relevance. Trialists and systematic reviewers have assumed that their focus on primary outcomes such as respiratory scales and hospital admission are important for families, clinicians and health services. However, bronchiolitis is a condition at a crossroads of disease severity, pre-existing comorbidities, clinical specialties and health care settings, and it is likely that perspectives vary between different stakeholders. This is reflected, for example, in the heterogeneous choice of primary outcomes between systematic reviews. Thus guidance and consensus are needed to select outcome domains that are relevant to various stakeholders, particularly caregivers of children with bronchiolitis. Further, measurement instruments chosen to measure these outcome domains are discrepant, as are the methods used to analyze resulting scores. Work is needed to define a minimum core set of clinically important efficacy and safety outcome domains, instruments and timing of assessments, for trials and systematic reviews in this field. This will contribute to a rational choice of primary outcomes for the trial, balancing outcome importance, rigorous measurement and power and feasibility issues, while preserving the choice to assess other outcomes that are relevant to the intervention and the condition.

3.2 MEASUREMENT PROPERTIES OF THE RDAI AND RDAI SCALES IN BRONCHIOLITIS

Adapted from:
Fernandes RM, Plint AC, Terwee CB, Sampaio C, Klassen TP, Offringa M, van der Lee JH. Validity of Bronchiolitis Outcome Measures. Pediatrics, in press.

Abstract form:
Ricardo M. Fernandes, Amy C. Plint, Caroline B. Terwee, Terry P. Klassen, Martin Offringa, and Johanna H. van der Lee. Measurement properties of RDAI and RACS and their suitability as outcome measures in bronchiolitis trials. European Respiratory Journal 2014 44:Suppl 58, P1256

Authors

Ricardo M Fernandes (1,2), Amy C Plint (3), Caroline B Terwee (4), Cristina Sampaio (2), Terry P Klassen (5), Martin Offringa (6), Johanna H van der Lee (7)

Affiliations

1. Department of Pediatrics, Santa Maria Hospital, Lisbon Academic Medical Centre, Lisbon, Portugal
2. Clinical Pharmacology Unit, Instituto de Medicina Molecular, University of Lisbon, Lisbon, Portugal
3. University of Ottawa and Children's Hospital of Eastern Ontario, Ottawa, Canada
4. Department of Epidemiology and Biostatistics and the EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, Netherlands
5. Manitoba Institute of Child Health, University of Manitoba, Winnipeg, Canada
6. Child Health Evaluative Sciences, Hospital for Sick Children, Toronto, Canada
7. Division of Woman and Child, Pediatric Clinical Research Office, Academic Medical Centre, Amsterdam, The Netherlands

ABSTRACT

Background

The Respiratory Distress Assessment Instrument (RDAI) and Respiratory Assessment Change Score (RACS) are frequently used in bronchiolitis clinical trials, but evidence is limited on their measurement properties. We investigated their validity, reliability and responsiveness.

Methods

We included data from up to 1765 infants with bronchiolitis enrolled in two studies conducted in pediatric emergency departments (ED). We assessed RDAI construct validity by testing hypotheses of associations with physiologic measures (respiratory rate, SatO2) and with constructs related to hospitalization, using correlation coefficients and multivariable analysis. RDAI/RACS responsiveness was evaluated using anchors of change based on these constructs; measures of responsiveness included the area under the curve (AUC). RDAI test-retest agreement and inter-rater reliability were evaluated using limits of agreement (LoA) and Intraclass Correlation Coefficients (ICC).

Results

Baseline RDAI scores were weakly correlated with respiratory rate ($r=0.38$, $p<0.001$), and scores increased in lower SatO2 categories ($p<0.001$). Higher RDAI scores were associated with hospitalization (OR 1.36 [1.26-1.47]); scores differed between participants that were discharged, admitted or stayed in ED ($p<0.001$). Our hypotheses were met, but the magnitude of associations was below our predefined thresholds. RDAI test-retest LoA were -3.80 to 3.64 (20% of the range), while inter-rater reliability was good (ICC=0.93). Formulated hypotheses for responsiveness were confirmed, with moderate responsiveness (AUC: RDAI 0.64 – 0.70; RACS 0.72).

Conclusions

RDAI has poor to moderate construct validity, with good discriminative properties but considerable test-retest measurement error. RDAI and RACS are responsive

measures of respiratory distress in bronchiolitis, but do not encompass all determinants of disease severity.

BACKGROUND

Acute viral bronchiolitis is the most common lower respiratory tract infection in infants and carries substantial clinical and financial burden.[2,795] There is wide practice variation in its management, with heterogeneous evidence for many therapeutic approaches.[8,10,81,405,775] Systematic reviews have highlighted various shortcomings in RCTs in this field (Chapter 2).[589,654] One of the major issues is the heterogeneous choice of outcome measures. There has been inconsistency in selected measurement instruments, whose measurement properties have often not been adequately studied (Chapter 2).[693]

Respiratory status is an important dimension and determinant of severity in bronchiolitis. The Respiratory Distress Assessment Instrument (RDAI) and Respiratory Assessment Change Score (RACS) are often used to measure this domain in bronchiolitis (Chapter 2). Lowell et al first described them in an RCT of epinephrine in wheezing infants.[714] The RDAI includes items on retractions and wheezing, while the RACS is a change score based on RDAI and respiratory rate. Evidence is limited regarding RDAI and RACS measurement properties and their suitability for use as evaluative instruments in clinical trials.[694,715,716] Previous RCTs have reported some data on which reliability and validity can be assessed, while the first formal validation study is recent.[717]

The aim of this study was to assess and compare the measurement properties of RDAI and RACS, i.e. validity, reliability and responsiveness.

METHODS

Population

We used data from two related studies conducted simultaneously in eight Canadian pediatric EDs during three bronchiolitis seasons (2004-2007): a 2x2 factorial RCT (Canadian Epinephrine/Steroid Trial, CanBEST; n=800); and a prospective cohort study (n=1554 infants, 584 of which also participated in CanBEST).[46] Both studies included infants <12 months with acute bronchiolitis (first episode of wheezing)

and excluded those with previous asthma, wheezing or use of bronchodilators. Additional exclusion criteria in CanBEST were: prematurity with corrected age <6 weeks, chronic cardiopulmonary disease or immunodeficiency, recent corticosteroid use or exposure to varicella, very mild or severe distress (pulse rate >200 beats/minute, respiratory rate >80 breaths/minute, or RDAI score <4 or >15) or lethargy.

Participants in CanBEST were randomly assigned to receive oral dexamethasone or placebo, and nebulized epinephrine or placebo, both administered in the ED (Figure 3.4). During the first 90 minutes only supplemental oxygen or acetaminophen were allowed. Other participants in the cohort study were given standard treatment as decided by the attending physicians.

In both studies written informed consent was obtained from the parents or guardians of the infants and both were approved by ethics committees at each site and by Health Canada.

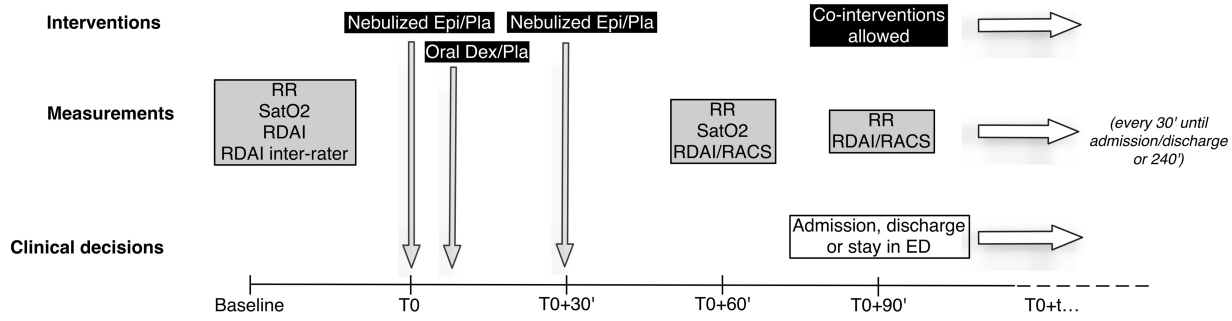


Figure 3.4. Timing of intervention, measurements and clinical decisions in the CanBEST trial (Dex, dexamethasone; Epi, epinephrine; Pla, placebo; RR, respiratory rate)

Instruments and outcome measures

We assessed the RDAI as described by Lowell et al, and a modification of the RACS as reported by Schuh et al (Table 3.3).[714,747] The following measurements were performed at baseline for both studies, and every 30 min until admission/discharge or 240 min for CanBEST: RDAI, respiratory rate, heart rate, oxygen saturation (SatO2), and activity status (Figure 3.4). Fever was also assessed at baseline. Research nurses performed all measurements after formal training and using written

Table 3.3: The Respiratory Distress Assessment Instrument (RDAI) and the Respiratory Assessment Change Score (RACS)

RDAI: Respiratory Distress Assessment Instrument*

Variable		Score					Range
		0	1	2	3	4	
Wheezing (auscultation)							
	Expiration	None	End	1/2	3/4	All	0-4
	Inspiration	None	Part	All			0-3
	Location	None	Segmental: ≤2 of 4 lung fields	Diffuse: ≥3 of 4 lung fields			0-2
	Partial sum score						0-8
Retractions (visual assessment)							
	Supraclavicular	None	Mild	Moderate	Marked		0-3
	Intercostal	None	Mild	Moderate	Marked		0-3
	Subcostal	None	Mild	Moderate	Marked		0-3
	Partial sum score	None	Mild	Moderate	Marked		0-9
Sum score (higher score indicates more severe disease)							0-17

Respiratory Assessment Change Score (RACS)

Variable	Formula	Range
Wheezing change score	Final partial sum score-baseline partial sum score	-8 - +8
Retractions change score	Final partial sum score-baseline partial sum score	-9 - +9
Respiratory rate "standardized change score	5% change: 0 units 6-15% change: -1/+1 units 16-25% change: -2/+2 units etc	-n - +n
Sum score (negative change scores indicate improvement)		-17-n - +17+n

*the original RDAI as reported by Lowell et al also included respiratory rate, i.e. it did not differentiate between the RDAI and RACS, and only used RACS as an outcome measure

#as modified by Schuh et al (2002); in the original RACS as reported by Lowell et al, final scores were substracted from baseline scores (i.e. positive change scores indicated improvement), and cut-offs to define respiratory rate change were defined at 10% intervals

instructions. SatO2 was measured by using pulse oximeters available locally. In both studies, the attending physician independently determined whether to admit or discharge the infant; RDAI was not used clinically at any site. In CanBEST physicians and nurses were blinded to treatment interventions, and by protocol any decisions regarding admission, discharge or continued stay in the ED was only to be made after the study interventions (i.e. after 90 min).

Statistical analysis

We used COSMIN's definitions of measurement domains and properties regarding validity, reliability and responsiveness.[701]

1. Construct validity of RDAI

There is no "gold standard" to assess bronchiolitis severity or respiratory distress. We assessed construct validity of the RDAI by formulating hypotheses about the direction and magnitude of the association of RDAI scores with both physiologic measures (respiratory rate, SatO2) and clinical decision-making constructs (decision to admit/discharge, and time to admission/ discharge).[671] We studied both convergent and discriminative validity.

We hypothesized that baseline RDAI scores and respiratory rate would have a strong positive correlation (Pearson's r $\geq$0.7). We used multiple linear regression analysis to explore possible confounding of this association, by activity status and fever (data from both studies), and age and weight (data from CanBEST). We further hypothesized a negative association between RDAI and SatO2, which we expected to be weaker and non-linear (Spearman r $\leq$-0.5), and we compared RDAI scores between three categories of SatO2 (<92%, 92-95%, >95%) (data from both studies).

We hypothesized that a higher RDAI score would increase the risk of admission (expected odds ratio for admission (OR) $\geq$1.5 for RDAI scores above the median). For this analysis, we used the last RDAI score assessed or registered before the time of admission/discharge (data from CanBEST). Multiple logistic regression analysis was used to evaluate whether that association was confounded by centre, treatment group, age and SatO2. Further, we expected participants that stayed in the ED longer to have intermediate scores as compared to those that were admitted or discharged sooner (data from CanBEST after the trial main interventions).

2. Reliability of RDAI

For RDAI test-retest reliability we considered that the group of CanBEST participants who had received both placebo interventions was stable between the 90- and 120-minute measurements. The same research nurse assessed the same child unblinded to the previous assessment. In a convenience sample of participants from each

study, two nurses performed baseline RDAI measurements independently in order to obtain inter-rater assessments.

For both test-retest and inter-rater conditions, we distinguished measures of measurement error from reliability measures.[796] To evaluate measurement error we calculated the standard error of measurement (SEM), the smallest detectable change (SDC), and we obtained a Bland-Altman plot and the 95% limits of agreement (LoA) (formulas in Appendix A3). The Bland and Altman Plot shows the mean differences between the test and retest scores (expressed in the unit of the scale) along the range of the scale. The LoA show the scores where 95% of these differences lay between. We assessed reliability by calculating the Intraclass Correlation Coefficient (ICC) in a two-way random effect model, including patient, time, and residual variance components (formulas in Appendix A3).

3. Responsiveness of RDAI / RACS

As with validity, we studied the responsiveness of the RDAI and RACS through testing hypotheses concerning the expected associations of change scores.[671] There is no clear criterion for change in bronchiolitis and none of the studies included explicit assessments of change. We based our hypotheses on physiologic and clinical constructs of change using a-priori-defined criteria to identify groups of participants that improved, versus those who had kept stable or deteriorated, irrespective of interventions. Criteria were based on respiratory rate and SatO2, and we used combined group data from CanBEST (Table 3.4).

We used different measures of responsiveness assessing statistical change or clinically important change, focusing on comparing the improved group with the stable/deteriorated group.[706] These included: testing differences in RDAI change scores and/or RACS within and between groups, and calculating standardized/ Cohen's effect size (ES) and the responsiveness ratio (formulas in Appendix A3). We hypothesized that patients who had improved would have larger change scores and effect sizes than patients who had not improved. We also used the area under the curve (AUC) of the ROC curve of improved vs stable groups (AUC>0.70 considered appropriate).

Table 3.4: Constructs of change and criteria used to assess responsiveness of RDAI and RACS

Anchor	Population		Timing of measurements	Criteria for change			
	Inclusion	n		Improved group		Stable/ deteriorated group	
				Criteria	n	Criteria	n
Change in respiratory rate (relative change)*	All participants	796	At baseline and last measurement before admission or discharge	≥25% reduction in respiratory rate	204	≤25% reduction in respiratory rate	592
Change in respiratory rate (tachypnea)*	Participants with tachypnea (baseline respiratory rate above 50 breaths/minute [<6 months] or above 40 breaths/minute [6-12 months])	305	At baseline and last measurement before admission or discharge	Reduction in respiratory rate below tachypnea cut-off	96	No reduction in respiratory rate below tachypnea cut-off	209
Probability of admission at baseline (versus actual decision)	Participants with high baseline probability of admission (respiratory rate >60 breaths/ minute or SatO2 <90%)	209	At baseline and last measurement before admission or discharge	Discharge	154	Admission	55

*Only for RDAI; not used to measure RACS responsiveness since respiratory rate is included in the RACS formula

For all analyses, we excluded participants with non-valid or missing data, with no imputation. Statistical significance was set at $p<0.05$ and we calculated 95% confidence intervals (95%CI) when applicable. We used SPSS version 19 (IBM SPSS Statistics, IBM Corporation, Armonk, NY).

Results

Figure 3.5 shows data sources and participants included in the analysis of each measurement property. The baseline characteristics of participants and selected outcomes from both studies are presented in Table 3.5. Participants in CanBEST were older than those in the cohort study, while baseline severity was greater in the latter.

1. Construct validity of RDAI

We found a weak positive correlation between RDAI score and respiratory rate at baseline with data from both studies - Pearson's r=0.38 [95%CI 0.35–0.45] ($p<0.001$) (n=1765). Correlations for retractions and wheezing sub-scores were r=0.41 and r=0.17, respectively. Using simple linear regression the coefficient

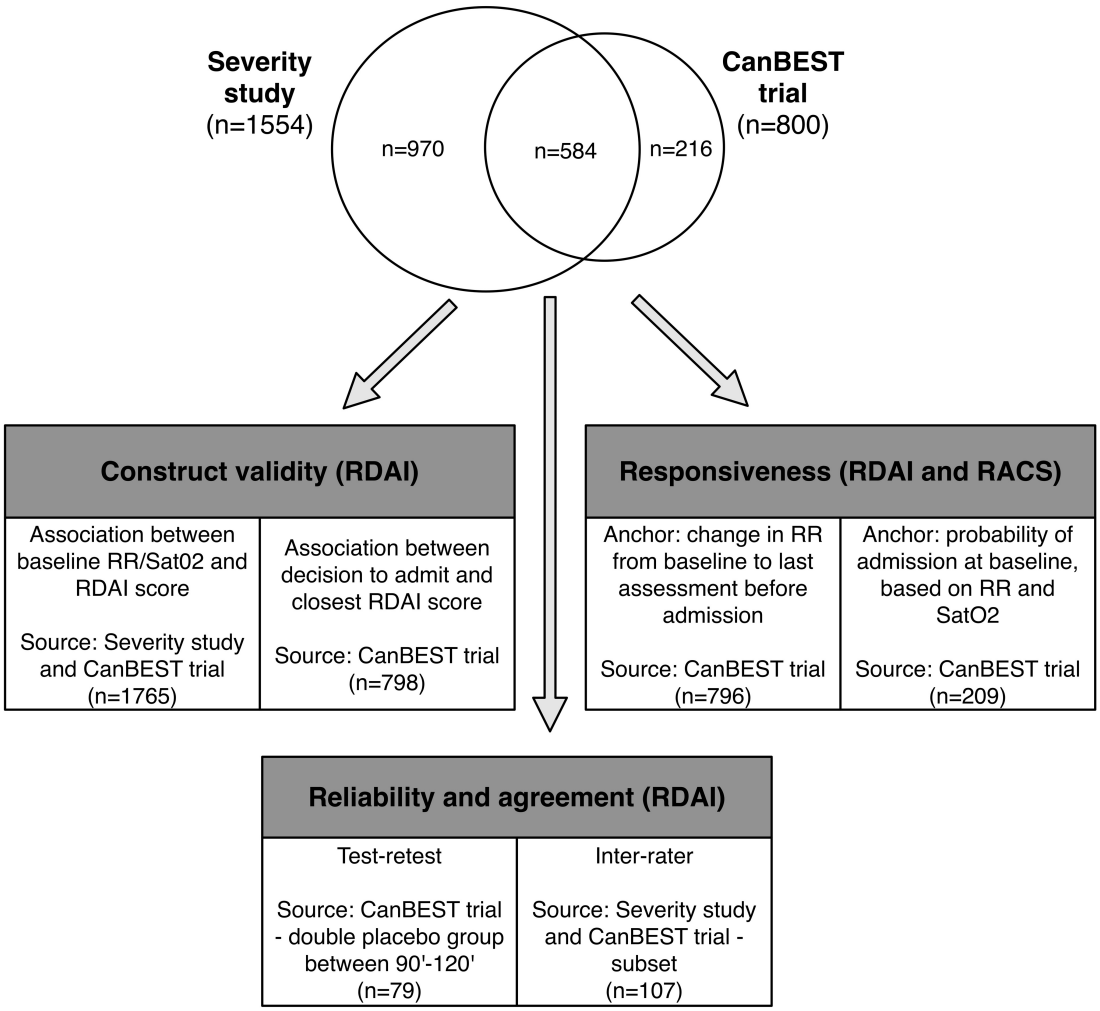


Figure 3.5. Sources of data and number of participants included in the analysis of each measurement property (RR, respiratory rate)

estimate was 1.55 [95%CI 1.38–1.73] increase in respiratory rate (breaths/minute) per increase in RDAI unit (p<0.001). The estimate was comparable when adjusting for fever and activity status (adjusted estimate 1.52). When restricting the analysis to CanBEST data, the correlation was weaker (Pearson's r=0.22, unadjusted linear regression estimate 0.98, n=800). The association was not confounded by age, weight, fever or activity status (adjusted estimate 0.92).

There was a weak negative correlation between baseline RDAI scores and SatO2 levels - Spearman's r=-0.24 (p<0.001) (n=1761). Correlations for retractions and wheezing were r=-0.25 and r=-0.14, respectively. RDAI scores increased in lower SatO2 categories (Figure 3.6). The median [interquartile range] RDAI scores were

Table 3.5: Baseline characteristics of participants and selected outcomes from the CanBEST trial and the cohort study

Baseline characteristics		CanBEST trial (n=800)*	Cohort study (n=1554)*
Demographics			
	Age – months, median [IQ range]	5 [3-7]	4 [2-7]
	Male gender – no. (%)	493 (62)	948 (61)
	Caucasian – no. (%)	654 (82)	1243 (80)
History			
	Personal history of atopy – no. (%)	89 (11)	157 (10)
	Prematurity – no. (%)	83 (10)	202 (13)
	Household smoking – no. (%)	305 (38)	575 (37)
	Symptom length – days, median [IQ range]	4 [2-5]	4 [2-5]
Clinical characteristics			
	Respiratory rate – breaths/minute, median [IQ range] ; >60 breaths/minute – no. (%)	48 [42-58]; 196 (25)	48 [42-60]; 441 (28)
	Oxygen saturation – % median [IQ range]; <90% – no. (%)	97 [95-98]; 24 (3)	97 [95-98]; 121 (8)
	Heart rate – beats/minute median [IQ range]; >180 beats/minute – no. (%)	150 [139-160]; 33 (4)	152 [140-164]; 143 (9)
	RDAI score – median [IQ range]; >12 – no. (%)	8 [6-10]; 76 (10)	8 [6-10]; 211 (14)
Patient outcomes			
	<90 minutes	103 (13)	NA
	90-120 minutes	261 (33)	NA
	120-240 minutes	248 (31)	NA
	>240 minutes	188 (23)	NA

IQR=interquartile range; NA=not applicable

*Data from the severity study include n=584 participants also included in CanBEST.

10 [8–12], 8 [6–10], and 7 [5–10] for SatO2 <92%, 92-95% and >95%, respectively (Kruskal Wallis test: p<0.001).

We found an association between the decision to admit or discharge and the last RDAI score of CanBEST participants. The preceding RDAI score was higher in admitted patients than in those who were discharged (mean difference 2.28 [95%CI 1.75–2.81] (t-test p<0.001) (n=798). A higher RDAI score was associated with

higher risk of admission (OR 1.36 [95%CI 1.26–1.47] per increase in RDAI unit and 2.54 [95%CI 1.65–3.92] when RDAI>8). Adjusted analyses for centre, treatment group, age and SatO2 found no relevant changes in these associations.

In addition, we found that RDAI scores measured after CanBEST interventions differed between the groups of participants that were discharged (median [interquartile range] 5 [2–6]), hospitalized (8 [5–10]) or those that stayed in the ED (6 [4–8]) (n=695, Kruskal Wallis test: p<0.001) (Figure 3.7). Differences between discharged participants and the two latter groups were statistically significant (Bonferroni posthoc: p=0.01 and p<0.01, respectively). Patients with a higher RDAI score at 90 minutes had higher risk of ED stay >240 min (OR 1.33 [95%CI 1.24–1.43] per increase in RDAI unit).

Overall, while results were in accordance with our validity hypotheses, the magnitude of the associations was mostly below our predefined thresholds.

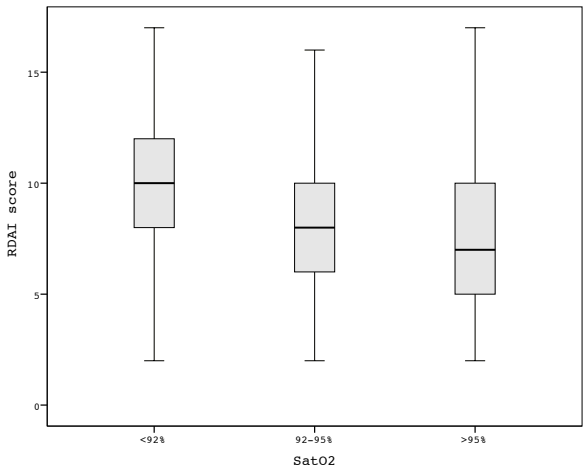


Figure 3.6. Box plot displaying baseline RDAI scores by categories of SatO2 (The box spans the interquartile range (IQR), the solid horizontal line through the box is the median value, and the whiskers denote values within 1.5 IQRs lower than the first quartile and 1.5 IQRs higher than the third quartile)

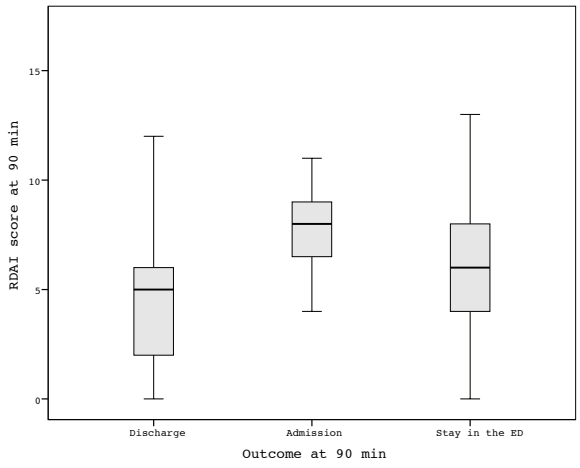


Figure 3.7. Box plot displaying RDAI scores and clinical decisions at 90 minutes (The box spans the interquartile range (IQR), the solid horizontal line through the box is the median value, and the whiskers denote values within 1.5 IQRs lower than the first quartile and 1.5 IQRs higher than the third quartile)

2. Reliability of RDAI

Test-retest assessments were available from 79 CanBEST participants. The mean difference between the two repeated assessments was 0.08 [95%CI -0.35–0.5]

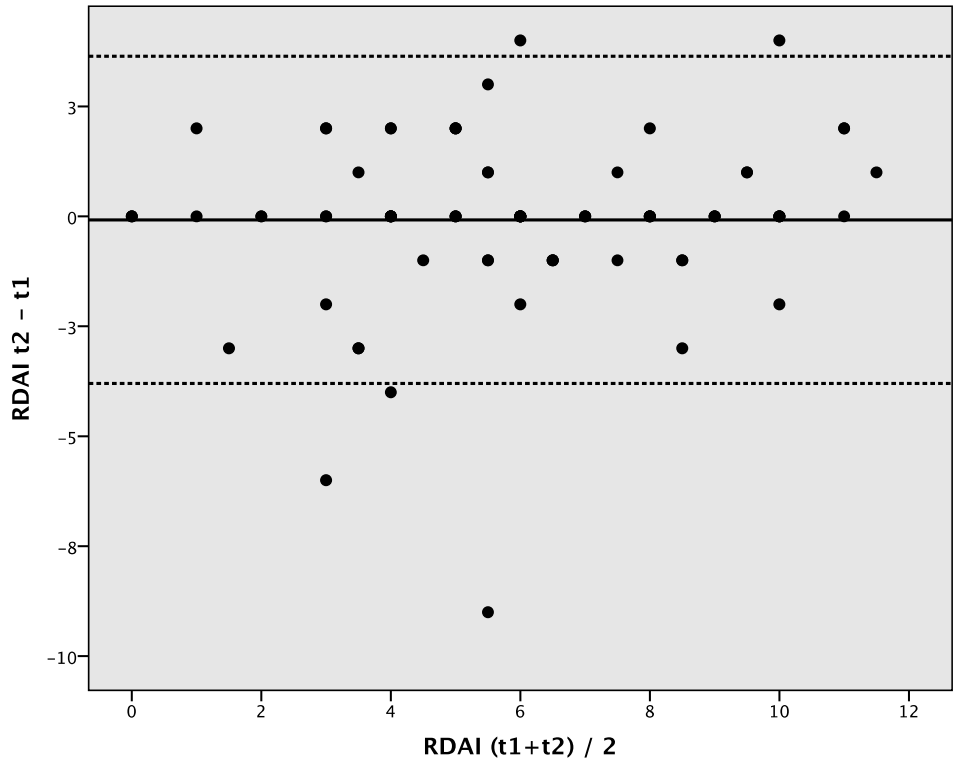


Figure 3.8. Bland and Altman plot of the difference between test-retest RDAI scores at t1 (90 min) and t2 (120 min) plotted against the mean value of both scores. The central line corresponds to the average difference between two RDAI scores (which reflects systematic error), whereas the lower and upper dotted lines correspond to lower and upper 95% limits of agreement (which reflect random error), respectively)

(p=0.72). The SEM was 1.34 and the SDC was 3.72 RDAI units. The test-retest 95% LoA were -3.8 to 3.64 RDAI units [95%CI -4.53 – -3.07, to 2.91 – 4.37]. This finding means that if a child is assessed twice, the second score could be between 3.64 points lower and 3.8 points higher than the first score, just because of measurement error. The magnitudes of differences between repeated measurements remained the same over the whole range of mean values as shown in the Bland-Altman plot (Figure 3.8). The test-retest ICC was 0.80 [95%CI 0.70–0.87].

Inter-rater assessments were performed in 107 participants. There was no significant difference between two repeated assessments – mean difference -0.06 [95%CI -0.28–0.15] (p=0.54). The SEM was 0.78, and the inter-rater LoA were -2.1 to 2.22 RDAI units [95%CI -2.46 – -1.74, to 1.86 – 2.58]. The ICC was 0.93 [95%CI 0.9-0.94].

3. Responsiveness of RDAI and RACS

Measures of responsiveness for RDAI and RACS based on the different constructs of change are presented in Table 3.6. By using both anchors, the mean RDAI scores decreased in both improved and stable groups (paired t-test p<0.001, for all within-group comparisons), with larger mean changes in scores of the improved group (unpaired t-test p<0.001, for all between-group comparisons). These results were in accordance with our predefined hypotheses. Between-group differences in mean RDAI change scores ranged from -1.31 [95%CI -1.85 – -0.77] for the 25% respiratory rate reduction criterion to -2.03 [95%CI -2.9 – -1.16] for the probability of admission criterion. Standardized ES for the improved group ranged from 1.43 to 1.71, while responsiveness ratios ranged from 1.54 to 1.61, and AUCs from 0.64 to 0.7. The RACS was larger in the improved group (between-group difference -2.81 (95%CI [-3.92 to -1.7]), with a responsiveness ratio of 1.96 and AUC of 0.72.

Table 3.6: Measures of responsiveness for RDAI and RACS using different anchors of change

Responsiveness measure		Anchor: respiratory rate 25% reduction (n=796)	Anchor: respiratory rate tachypnea cut-off (n=305)	Anchor: probability of admission (n=209)	
		RDAI	RDAI	RDAI	RACS
Within-group mean change (RDAI) or change score (RACS) ± SD					
	Improved group	-4.17 ± 2.86	-4.48 ± 3.07	-3.63 ± 2.96	-5.94 ± 3.76
	Stable group	-2.86 ± 2.71	-2.8 ± 2.78	-1.6 ± 2.36	-3.13 ± 3.03
Between-group difference in mean change (RDAI) or change score (RACS) [95% CI]		-1.31 [-1.85 to -0.77]	-1.67 [-2.37 to -0.97]	-2.03 [-2.9 to -1.16]	-2.81 [-3.92 to -1.7]
Standardized ES					
	Improved group	1.54	1.71	1.43	NA
	Stable group	1.22	1.15	0.67	NA
Responsiveness ratio		1.54	1.61	1.54	1.96
Area Under the Curve [95% CI]		0.64 [0.59-0.68]	0.65 [0.59-0.71]	0.7 [0.63-0.78]	0.72 [0.64-0.8]

Measurements were made at baseline and before admission or discharge. NA, not applicable

Discussion

This study of measurement properties of RDAI and RACS in acute bronchiolitis identifies strengths and limitations of their use as outcome measures. The RDAI was evaluated in three systematic reviews of measurement properties of asthma or wheezing severity scales in children.[694,715,716] Limited data on its reliability and responsiveness were provided in the original description of the scale, and in later reports of RCTs.[714,747,797] However, none of these were adequately designed measurement studies, and no formal assessment of validity was found. Destino et al recently reported the first validation study on RDAI in bronchiolitis, showing poor construct validity, inter-rater reliability and responsiveness.[717] Findings on validity were fairly consistent with our results; differences in setting, raters and methods may explain why results on reliability and responsiveness were distinct.

Our results show that the RDAI has poor to moderate construct validity. The RDAI was developed ad hoc with no elaboration on the underlying conceptual model, item selection, scoring or weighting. While in the original report only the RACS was used as an outcome measure, later trials used RDAI scores separately for single or repeated assessments.[714] In our conceptual framework respiratory distress was putatively reflected by RDAI items (i.e. reflective model) and contributed to the multidimensional construct of bronchiolitis.[671] We found poor convergent validity with respiratory rate, but RDAI scores discriminated well between clinically meaningful SatO2 subgroups. Measurement properties from other respiratory scales or their individual items, which often include respiratory rate or SatO2, are seldom available.[694,715,716] When they are reported, there is substantial heterogeneity in correlations with SatO2, ranging from poor to moderate. Thus, our predefined cut-offs may have been too strict. Most, but not all, studies are consistent with our findings of weaker correlations between SatO2 and auscultatory items when compared to work of breathing.[694,715,716,798]

These results reflect the pathophysiology and clinical correlates of respiratory distress in bronchiolitis. It is known that, as disease progresses and severity increases, so do the disturbances in ventilation and ventilation-perfusion matching.[144,246] Many patients have effective compensatory mechanisms for these disturbances, while others do not. However, clinical signs of respiratory distress may not capture hypoxemia/hypercapnia balance equally. Furthermore, the

correlation between SatO2 (reflecting oxygenation), and respiratory rate (also dependent on respiratory drive and ventilation), varies across conditions.[798] Therefore, the RDAI likely does not represent all dimensions of respiratory distress in bronchiolitis, and a combination of parameters may be more relevant for the measurement of respiratory distress, as seen in formally developed scales.[799,800] However, most other scales were not developed specifically for bronchiolitis, and their measurement properties cannot be transferred between different respiratory conditions without further validation.

We found that the RDAI had reasonable predictive validity based on its association with hospitalization and length of stay in the ED. Our findings are consistent with those of Corneli et al, who identified RDAI score, SatO2, and respiratory rate as predictors of hospitalization in bronchiolitis.[352] On the contrary, Destino et al found that RDAI sum scores did not discriminate well between admitted and discharged patients, but the item on retractions did.[717] Two large prognostic studies have also identified retractions as predictors of severe disease in ED and hospitalized patients.[68,351] Decisions regarding hospitalization and length of stay in the ED are multifactorial. Non-respiratory severity parameters (e.g. feeding), prognostic factors (e.g. age), social issues, clinical judgment, available resources and local practices influence decision-making.[352] Further, there are limits to the validity of static measurements of respiratory distress in a highly dynamic condition. From an outcome measure perspective, RDAI does not encompass all determinants of bronchiolitis severity.

Inter-rater reliability measured by the ICC was good, both at group and individual level, as was inter-rater measurement error. These findings means that RDAI scores can adequately discriminate participants assessed by different raters at the same time point in both clinical and research settings. Data from previous RCT reports also showed good inter-rater reliability, but Destino et al found a strikingly low ICC.[717] Differences may relate to training, familiarity with the instrument, raters and population heterogeneity. On the other hand, we found considerable test-retest measurement error at individual level, since a patient should change at least close to 4 points (about one-fifth of the scale) before a change is detectable beyond measurement error. Thus in clinical practice, changes in individual patients should be interpreted with caution. For the RACS, we must also consider measurement

error for respiratory rate.[366,369] The SDC is paramount to interpretability parameters such as the minimal important change (MIC), since a large SDC relative to the MIC means that observed change may be caused by measurement error rather than change per se.[709] At the individual level, taking repeated measurements and averaging the value would reduce the measurement error with a factor √k (k is the number of measurements). Although reassessment is a key component when evaluating children with respiratory distress, many repeated measurements might not be practical in clinical practice. At the group level, the SDC of a mean change is equal to SDC/√n, which reduces its impact.[671] Since the ICC was high, the RDAI is reliable for use in studies. Overall, these results suggest that the RDAI has adequate discriminative properties, but test-retest measurement error should be minimized (e.g. through repeated measurements).

The RDAI was responsive according to our predefined hypotheses based on two distinct constructs of change. Previous data on RDAI responsiveness is scarce.[694,715,716] Hardly any intervention can be considered clearly effective in bronchiolitis in the ED setting, and thus none is a reasonable gold-standard to assess change. Destino et al reported a mild correlation between the change in the RACS and the Children's Hospital of Wisconsin Respiratory Score, but data on responsiveness of this latter scale is also missing.[717] We anchored our constructs of change on physiological change, and change in clinical status likely to be relevant for decisions regarding patient disposition at the ED. Measures of responsiveness that took into account both improved and stable groups (responsiveness ratio and AUC) were comparable between anchors for the RDAI. The AUC value was close to the frequently used cut-off of acceptability (0.7) for both the RDAI and RACS, with the RACS being slightly more responsive. This data suggest the RDAI and RACS are moderately responsive, but any comparison with other respiratory scales is limited.

Our study has limitations related to design constraints of both included studies. First, less heterogeneity of RDAI scores in the selected sample of CanBEST participants may explain why we found a weaker correlation with respiratory rate and lower test-retest ICC scores. Further validation is needed when considering children with very mild or severe disease, which were excluded in CanBEST. Second, our results are applicable to infants with a first episode of wheezing and no relevant co-morbidities, and should be interpreted with caution when defining

bronchiolitis differently in other populations.[4] Third, concurrent factors that affect decisions of hospitalization were not collected, and the exact timing of this decision was not known. While, in ideal conditions, managing physicians would be blinded to RDAI/RACS scores, blinding to their individual items is not expected. Finally, defining stability and change can be problematic and time-dependent due to the dynamic nature of bronchiolitis. When assessing responsiveness using data collected at different time points (mostly between 90 and 240 minutes), we observed significant improvements in RDAI scores in groups that we considered a priori to be stable. This is likely a limitation of our anchors, and may also reflect the effect of supportive measures and the nebulized "placebo". These limitations should be considered when calculating the MIC of the RDAI, which will be the focus of future work.

In conclusion, we found the RDAI to be an incomplete measure of respiratory distress in bronchiolitis, with poor to moderate construct validity and adequate inter-rater reliability. The RDAI had considerable test-retest measurement error, and while both the RDAI and RACS were moderately responsive, methodological issues may limit the interpretation of this finding. Finally, the RDAI does not encompass all determinants of bronchiolitis severity.

CHAPTER 4

PERSPECTIVES ON DEFINITIONS AND OUTCOMES IN BRONCHIOLITIS TRIALS

Submitted as:
Fernandes RM, Andrade GM, Constant C, Malveiro D, Magalhães M, Abreu D, Azevedo I, Sousa E, Salgado R, Bandeira T. Acute viral bronchiolitis: physician perspectives on definition and clinically important outcomes. Currently in review by Pediatric Pulmonology.

Abstract form:
Fernandes RM, Andrade GM, Constant C, Malveiro D, Magalhães M, Abreu D, Azevedo I, Sousa E, Salgado R, Bandeira T. Perspectives of pediatricians and general practitioners on definition and important outcomes in bronchiolitis. European Respiratory Journal 2014 44:Suppl 58, P1257

Authors

Ricardo M Fernandes (1,2), Maria Gabriela Andrade (2), Carolina Constant (1,3), Duarte Malveiro (4), Manuel Magalhães (5,6), Daisy Abreu (2), Inês Azevedo (5,7), Eduarda Sousa (4), Rizério Salgado (7), Teresa Bandeira (1,8)

Affiliations

1. Department of Pediatrics, Hospital de Santa Maria, Centro Académico de Medicina de Lisboa, Lisboa
2. Clinical Pharmacology Unit, Instituto de Medicina Molecular, Universidade de Lisboa, Lisboa
3. Department of Pediatrics , Unidade de Torres Vedras, Centro Hospitalar do Oeste, Torres Vedras
4. Department of Pediatrics , Centro Hospitalar Lisboa Ocidental, Lisboa
5. Department of Pediatrics , Faculty of Medicine, University of Porto, Porto
6. CINTESIS - Centre for Research in Health Technologies and Information Systems, Porto
7. EPIUnit, Institute of Public Health, University of Porto, Porto
8. Unidade de Saúde Familiar S. Julião, Centro de Saúde de Oeiras, Oeiras
9. Clínica Universitária de Pediatria, Faculdade de Medicina, Universidade de Lisboa, Lisboa

ABSTRACT

Background

Two key limitations hamper intervention research in bronchiolitis: the absence of a clear definition of disease, and the heterogeneous choice of outcome measures in current clinical trials. We assessed how pediatricians and general practitioners (GPs) perceived definition and clinically important outcomes in bronchiolitis.

Methods

A nationwide online survey (ABBA study) was conducted through the Portuguese Society of Pediatrics and GPs' mailing lists. We assessed agreement with statements on bronchiolitis definition, and participants were asked to score the relative importance of several outcomes. Principal component analysis (PCA) explored dimensions underlying disease definition. Outcomes were ranked by mean score and proportion given highest score.

Results

We included 514 pediatricians and 165 GPs (overall 59% were board-certified). Most pediatricians (76.5%) agreed with a definition based on coryza, wheezing and/or crackles/rales, compared to 38.1% GPs ($p<0.001$). Less than 5% physicians agreed with a definition commonly used in clinical trials (<12 months, first episode of wheeze). We retained three dimensions on PCA: one based on coryza, rales/crepitations and no sudden onset; another on number of episodes and age; and a third on wheeze. Dimensions varied by physician specialization and training ($p<0.01$). Hospital admission and respiratory distress were top rated outcomes by both groups of physicians.

Conclusion

Physician definitions of bronchiolitis have considerable variability and often mismatch those of clinical trials. Rating of important outcomes was consistent. Our results highlight the need for a robust standardized definition of acute bronchiolitis in infants and support the development of a core outcome set for future clinical trials.

BACKGROUND

Acute viral bronchiolitis is the most common lower respiratory tract infection in infants.[2] It entails substantial clinical and financial burden across different levels of care. Treatment is controversial, and there is wide practice variation and inconclusive evidence for many therapeutic approaches.[10,551] Systematic reviews in this field have highlighted two shortcomings in clinical trial design: the absence of a clear definition of disease, and the heterogeneous choice of outcome measures (Chapters 2 and 3.1).

While bronchiolitis is a relatively straightforward clinical diagnosis for most child health practitioners, no standardized set of diagnostic criteria exists.[2,4,19,719,720,721] A Delphi panel from a local guideline in the United Kingdom reported consensus on bronchiolitis as 'a seasonal viral illness characterized by fever, nasal discharge and dry, wheezy cough', with 'fine inspiratory crackles and/or high pitched expiratory wheeze'.[4] However, definitions may vary by auscultatory findings, age, number of episodes or other parameters.[95,214,726] Furthermore, the label 'bronchiolitis' may overlap with acute wheezing and asthma, which hampers the interpretation of current evidence.[465,764] Empirical data is lacking on physician's perspectives of definitions and their determinants.

The second issue relates to the inconsistency in reported outcome domains measured in bronchiolitis trials (Chapter 3.1).[693] This limits the interpretability of trial findings and the opportunity for pooling results in meta-analysis. Initiatives such as OMERACT and COMET have supported the development and use of agreed standardized collection of outcomes, i.e. core outcome sets, to be used, as a minimum, in all trials for a specific clinical area.[681,682] Core outcome sets should be meaningful for key stakeholders. However, there is scarce evidence on which outcomes in bronchiolitis are clinically relevant.

The ABBA survey aimed to assess the perspectives of physicians, across the nation, on definition of bronchiolitis, and on clinical importance of different outcomes in this field. We engaged and compared perspectives from paediatricians and general practitioners (GPs), as both groups are key stakeholders involved in the management of bronchiolitis of varied severity at different levels of care, and have distinct background and training that may influence these perspectives.

METHODS

Study design and subjects

The ABBA study was a cross-sectional electronic survey targeting pediatricians and GPs working in Portugal, both board-certified physician specialists and postgraduate physicians in a residency training program. Pediatricians were current members of the Portuguese Society of Pediatrics (SPP), which has an open membership across levels of care. Electronic contact information was provided by the organization, after review and approval of the research protocol. Subjects with invalid e-mail addresses were excluded. We reached GPs who were registered in any of three national GP mailing lists with the support of the Portuguese Association of General Practitioners (APMGF).

Questionnaire

A multidisciplinary panel from the Pulmonology section of the SPP and the APMGF designed a questionnaire in Portuguese on various topics of bronchiolitis; definition and outcomes are the focus of this paper. The instrument was developed using a structured approach. Based on a literature review and the panel's expertise, items were formulated for each construct and examined for face validity. Item selection and reduction was achieved through consensus. We obtained 18 items on definitions and outcomes, and we collected data on physicians' training and workplace (questionnaire available in appendix A4). Response formats included multiple choice items, ordinal ratings (1 to 5) and 5-point Likert items (ranging from disagree completely to agree completely).

First, we asked practitioners to express their level of agreement with six statements on key history and clinical findings in defining bronchiolitis. These included onset of symptoms, preceding coryza, auscultatory findings (crackles/rales and wheezing), upper age limit and maximum number of episodes. Regarding outcomes, participants were asked to score the importance given to a predefined list of 12 outcomes in the interpretation and applicability of clinical trial results. We included outcomes that have been previously reported in bronchiolitis trials (e.g. hospital admission, clinical severity), and added outcomes recognized as relevant but often missing in this field (e.g. quality of life, parent-reported symptoms). The survey was

developed using the SurveyMonkey platform (www.surveymonkey.com), and the instrument was pilot tested for acceptability and feasibility.

Implementation

The survey ran from 5th April to 22nd May 2013. A modified Dillman technique was employed to optimize the response rate, including up to four reminder e-mails and a small incentive. Pediatricians were contacted through personalized e-mail invitations, with a unique link that prevented multiple entries. An e-mail invitation with an open link was sent to each GP mailing list. Consent was implied by survey completion, and data was anonymized for analysis. The Ethics Committee of Centro Hospitalar Lisboa Norte/Faculdade de Medicina de Lisboa approved this study (approval statement available in appendix A4).

Preliminary data indicated approximately 1300 members of SPP from a universe of around 1750 pediatricians and 400 residents registered nationwide in 2011. About 1400 GPs were registered in all three mailing lists. Previous electronic surveys performed within smaller pediatric organizations reached response rates up to 60-75%. We anticipated 40-50% responses from pediatricians, but a lower rate from GPs due to the method recruitment.

Statistical analysis

Definitions of bronchiolitis

We stratified results by pediatricians and GPs, and compared responses to Likert items using the t-test. We evaluated whether physician perspectives were in agreement with two existing definitions, one based on a consensus approach for a local guideline, the other based on inclusion criteria used in recent randomized trials (operational criteria shown in Table 4.1), using the χ^2 test.[45,46,726]

We used exploratory factor analysis to examine whether any meaningful dimensions could be distinguished underlying the perspectives on definitions of bronchiolitis.[801] Principal component analysis (PCA) is based on item correlations; items that correlate highly with each other are clustered in one factor/component, and share variance explained by the underlying dimension. PCA aims to explain as much total variance with a minimal number of components. We performed PCA including data from all subjects on the six items on definition of bronchiolitis. We

determined the number of components to retain based on two criteria: magnitude of the eigenvalue >1 (main criteria) and examination of scree plot. Selected components were rotated to facilitate interpretation and to generate component loading scores, which measure the association between items and the underlying component. We used the varimax orthogonal rotation, since components did not show considerable correlation between each other (<0.2). Sampling adequacy was assessed through the Kaiser-Meyer-Olkin measure, and we used the Bartlett's test of sphericity to test for homogeneity. Items with conventional loading >0.4 (absolute value) were interpreted for each component. Using the resulting components as dependent variables, we used multivariate analysis of variance (MANOVA) to examine associations with group of physician (pediatricians vs. GPs), training (resident vs. specialist) and location (Lisbon and south vs. other regions).

Table 4.1: Operational criteria used for preexistent definitions of bronchiolitis

Nottingham guideline-based definition*	Clinical trial-based definition*
Agree or completely agree with 'coryza preceding symptoms' AND agree or completely agree with 'wheezing' OR with 'crackles/rales' on auscultation AND neutral or disagree or completely disagree with 'sudden onset of symptoms'	Agree or completely agree with 'wheezing on auscultation' AND 'first episode' AND '<12 months of age'
*answers to other parameters were not restricted	

Outcomes in bronchiolitis

We analyzed the distribution of scores given to each outcome by pediatricians and GPs, and identified outcomes that were rated 4 or 5 by over 80% of participants. Further, we ranked outcomes within each subject (for equal scores, standard competition ranking, i.e. '1224' was used). We then calculated for each outcome the proportion of participants giving it the highest score/ranking. Overall ranking of outcomes was based on this proportion, as well as on the highest mean scores.

For all analyses, participants with incomplete responses were excluded for the corresponding parameter, with no imputation of missing data. p values < 0.05 were

considered statistically significant. All analyses were performed using SPSS for Mac and Windows (SPSS Inc, version 21.0).

RESULTS

A flow diagram of participant recruitment is shown in Figure 4.1. We sent 1218 invitations to potentially eligible SPP members, and included 514 (response rate 44%). Of approximately 1400 GPs registered in mailing lists, 165 subjects participated (estimated 12% response rate). Complete responses were available from more than 90% pediatricians and more than 70% GPs. Table 4.2 summarizes the demographic characteristics of included participants. For both pediatricians and GPs the majority of responders were specialists (59.2%) and worked in Lisbon or north of Portugal (74.9%). Of pediatricians, 42.3% worked in all practice settings (ambulatory i.e. outpatient, emergency department and hospital), while most GPs provided pediatric ambulatory care (94.5%), and 29.7% also worked in pediatric emergency settings.

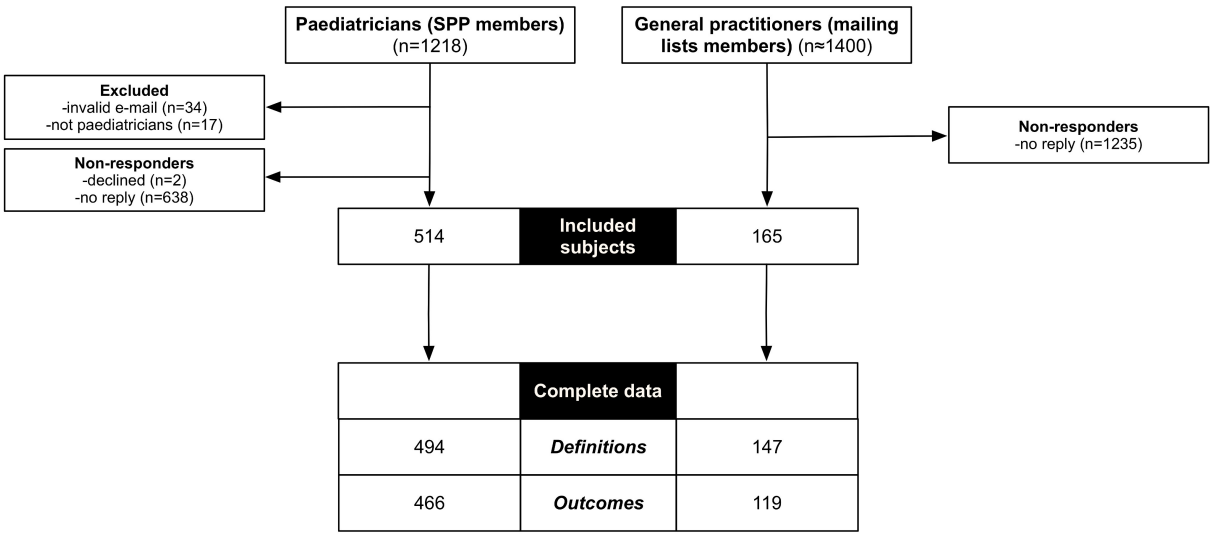


Figure 4.1: Study flowchart of paediatricians and general practitioners

Table 4.2: Demographic characteristics of paediatricians and general practitioners

Demographic characteristics		Paediatricians (n=514)*	General Practitioners (n=165)*
Level of training - n (%)			
	Resident	215 (42)	62 (38)
	Specialist	299 (58)	103 (62)
Time since graduation - median [P25-75] years			
	Resident	5 [4-6]	4 [3-5]
	Specialist	14 [10-25]	9.5 [7-24.5]
Practice setting - n (%)#			
	Ambulatory care	319 (63)	156 (95)
	Emergency department	432 (86)	49 (30)
	Hospital care	362 (72)	1 (1)
Workplace - n (%)†			
	Lisbon region	206 (41)	65 (41)
	North	175 (35)	50 (32)
	Center	80 (16)	34 (21)
	Other	42 (8)	10 (6)

*results were calculated based on the number of respondents to a particular question; data available from n=514 paediatricians and n=165 general practitioners unless specified

#data available from n=503 paediatricians; practice settings were not mutually exclusive

†data available from n=503 paediatricians and n=159 general practitioners

Definitions of bronchiolitis: descriptive statistics

The perspectives of pediatricians (n=494) and GPs (n=147) on key history and clinical findings are shown in Figure 4.2 (number of episodes and age), and Figure 4.3 and Table 4.3 (symptoms and signs). Most physicians on both groups agreed or agreed completely with presence of wheezing on auscultation (92.9% pediatricians and 95.2% GPs) (p=0.11). However, we found significant differences between groups on all other parameters (p<0.001 for all comparisons). Most pediatricians agreed or agreed completely with preceding coryza and presence of crackles/rales (92.5% and 89.5%, respectively), and disagreed or disagreed completely with sudden onset of symptoms (68%). Further, they most often restricted the diagnosis of bronchiolitis based on number of episodes (up to three episodes 47.3%, only first

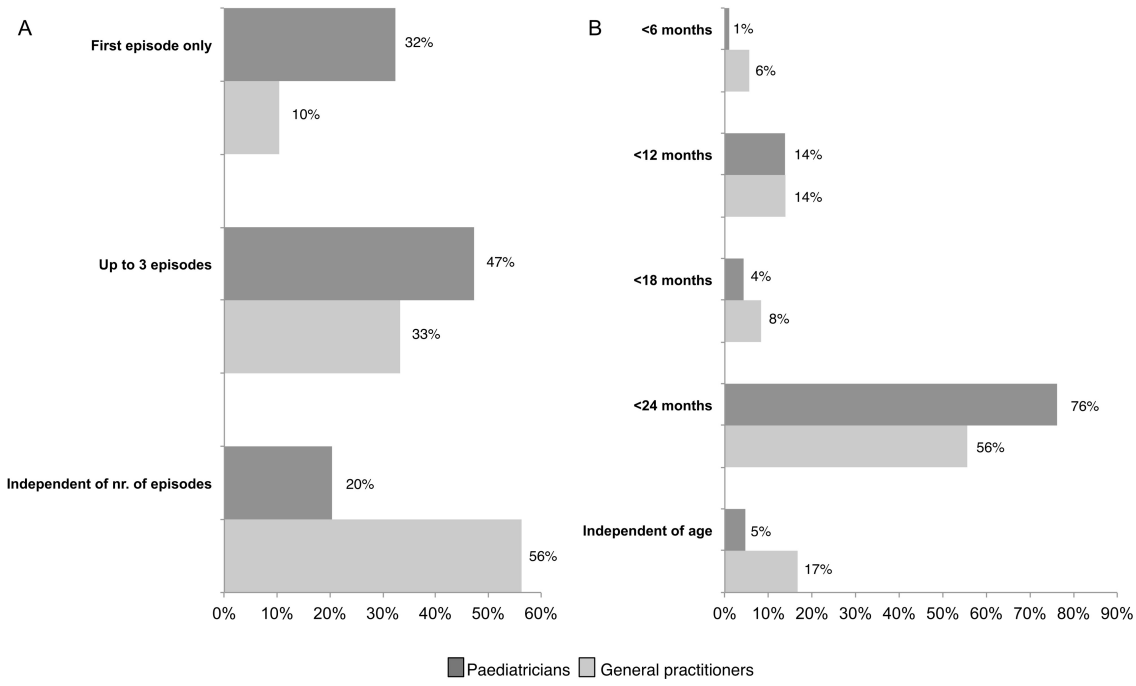


Figure 4.2: Perspectives of pediatricians and general practitioners on number of episodes (panel A) and age (panel B) limits for the definition of bronchiolitis

episode 32.4%), and upper age limit (up to 24 months 76.2%, up to 12 months 13.8%). On the contrary, 52.4% GPs agreed or agreed completely with sudden onset of symptoms, with a lower proportion than pediatricians agreeing or agreeing completely with preceding coryza and presence of crackles/rales (70.1% and 42.9%, respectively). Also, many GPs did not restrict bronchiolitis by number of episodes (56.3%) or age (16.7%).

A definition of bronchiolitis close to the Nottingham guideline had the agreement of 76.5% pediatricians and 38.1% GPs ($p<0.001$). A commonly used clinical trial definition of bronchiolitis (limited to a first episode of wheezing below 12 months) had the agreement of only 4.1% pediatricians and 2.8% GPs ($p<0.001$).

Table 4.3: Perspectives of pediatricians and general practitioners on key history and clinical findings in bronchiolitis

Clinical findings	Reported agreement with key findings					p-value*
	Disagree completely	Disagree	Neither agree nor disagree	Agree	Agree completely	
PEDIATRICIANS (n=494)						
Sudden onset of symptoms	83(17)	253(51)	58(12)	91(18)	9(2)	<0.001
Coryza preceding symptoms	4(1)	9(2)	24(5)	254(51)	203(41)	<0.001
Wheezing on auscultation	0	7(1)	28(6)	331(67)	128(26)	0,11
Rales or crepitations on auscultation	3(1)	23(5)	26(5)	328(66)	114(23)	<0.001
GENERAL PRACTITIONERS (n=147)						
Sudden onset of symptoms	14(9)	35(24)	21(14)	64(44)	13(9)	<0.001
Coryza preceding symptoms	2(1)	17(12)	25(17)	67(46)	36(24)	<0.001
Wheezing on auscultation	0	3(2)	4(3)	91(62)	49(33)	0,175
Rales or crepitations on auscultation	15(10)	42(29)	27(18)	56(38)	7(5)	<0.001

*p-value based on t-test comparing group means of each Likert item

Definitions of bronchiolitis: factor analysis

Principal component analysis was conducted with data from 644 participants: the correlation matrix between the six items is shown on Table 4.4, each component's eigenvalues and percentages of variance explained are shown on Table 4.5, and a scree plot on Figure 4.4. Three components that explained 63.9% of the variability were retained. The rotated component matrix is shown in Table 4.6. The first component correlated with 'coryza preceding symptoms' and 'crackles/rales on auscultation', but was inversely correlated with 'sudden onset of symptoms' (Principal Component 1 - PC1, defined as 'coryza and crackles/rales, no sudden onset'). The second component was determined by 'number of episodes' and 'age of the child' (PC2, 'age and episodes'), which varied together, with a stronger correlation with the latter. The third component was mostly correlated with 'wheeze on auscultation' (PC3, 'wheeze').

Table 4.4: Perspectives on definition of bronchiolitis: correlation matrix

	Sudden onset of symptoms	Coryza preceding symptoms	Wheezing on auscultation	Crackles/ rales on auscultation	Number of episodes	Age of the child
Sudden onset of symptoms	1,000	-,321	,140	-,192	,147	-,002
Coryza preceding symptoms	-,321	1,000	,140	,270	-,176	-,024
Wheezing on auscultation	,140	,140	1,000	-,024	,018	-,072
Rales or crepitations on auscultation	-,192	,270	-,024	1,000	-,169	,073
Number of episodes	,147	-,176	,018	-,169	1,000	,124
Age of the child	-,002	-,024	-,072	,073	,124	1,000

Table 4.5: Principal component analysis: eigenvalues and percentages of variance explained

Principal component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
PC1	1,650	27,504	27,504	1,650	27,504	27,504	1,603	26,723	26,723
PC2	1,151	19,177	46,681	1,151	19,177	46,681	1,128	18,798	45,522
PC3	1,035	17,248	63,929	1,035	17,248	63,929	1,104	18,408	63,929
PC4	,854	14,228	78,157						
PC5	,738	12,308	90,465						
PC6	,572	9,535	100,000						

Using MANOVA, we found significant associations of underlying components with physician group ($p<0.001$) and level of training ($p=0.003$), but not geographical workplace ($p=0.811$). In particular, pediatricians and residents assigned higher scores on PC1 (i.e. they placed more emphasis on this component), as compared to GPs and specialists, respectively ($p<0.001$). Compared to GPs, pediatricians scored lower on PC2 ($p=0.011$) and PC3 ($p=0.002$) (i.e. they placed less emphasis on these components).

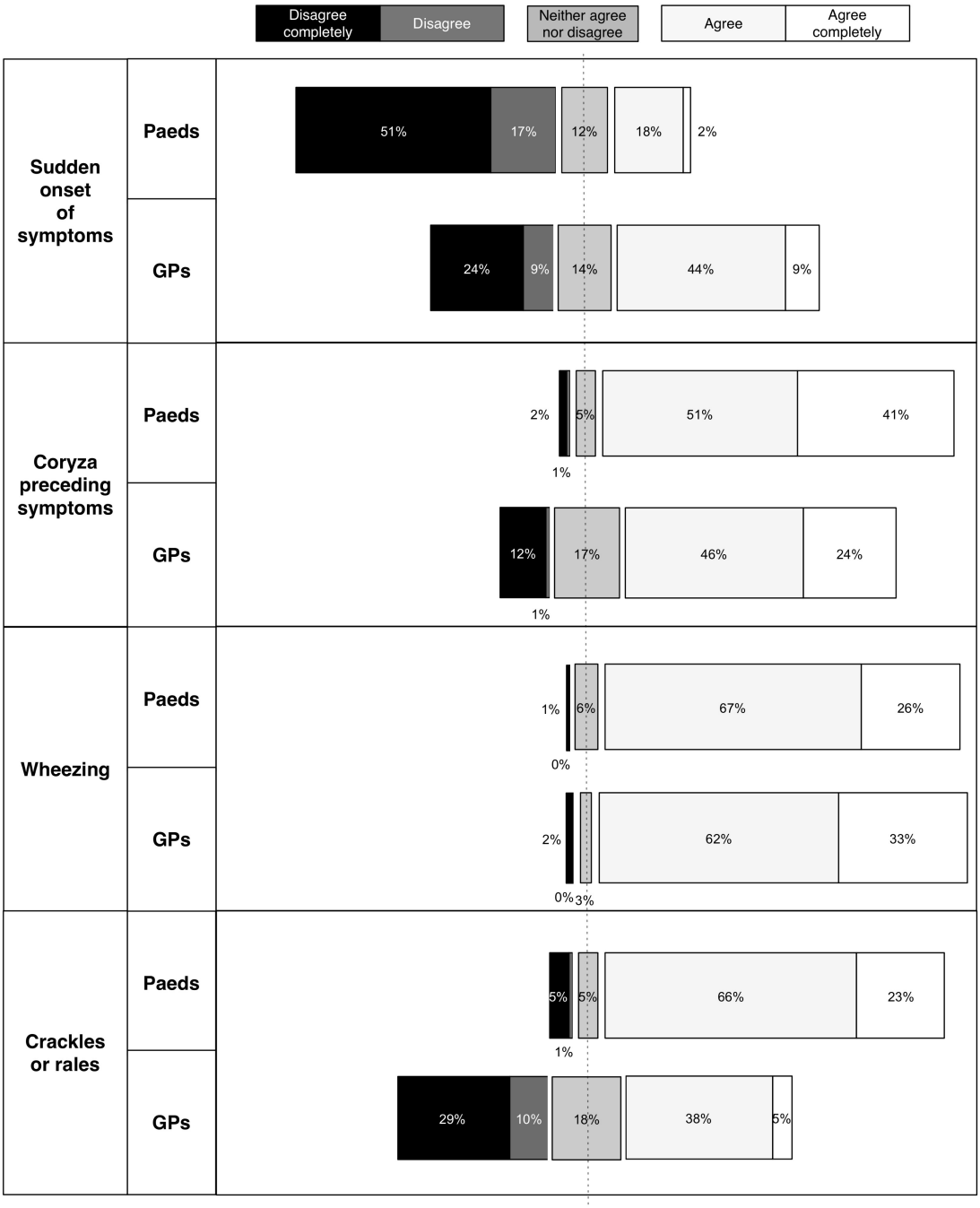


Figure 4.3: Perspectives of pediatricians and general practitioners on key history and clinical findings in bronchiolitis

Table 4.6: Perspectives on definition of bronchiolitis: principal component analysis with factor loadings, eigenvalues and explained variances*#

		Principal Components		
		PC1	**PC2**	**PC3**
Item				
	Sudden onset of symptoms	**-0,643**	0,125	0,327
	Coryza preceding symptoms	**0,743**	-0,112	0,298
	Wheezing on auscultation	0,021	-0,027	**0,938**
	Crackles/rales on auscultation	**0,685**	0,099	-0,010
	Number of episodes	-0,365	**0,599**	0,122
	Age of the child	0,188	**0,855**	-0,118
Eigenvalue before rotation		1,65	1,151	1,035
Explained variance (%)		26,723	18,798	18,408
Eigenvalue after rotation		1603	1128	1104

*Factor loadings of absolute value ≥0.40 are shown in bold.

#Rotation Method: Varimax with Kaiser Normalization.

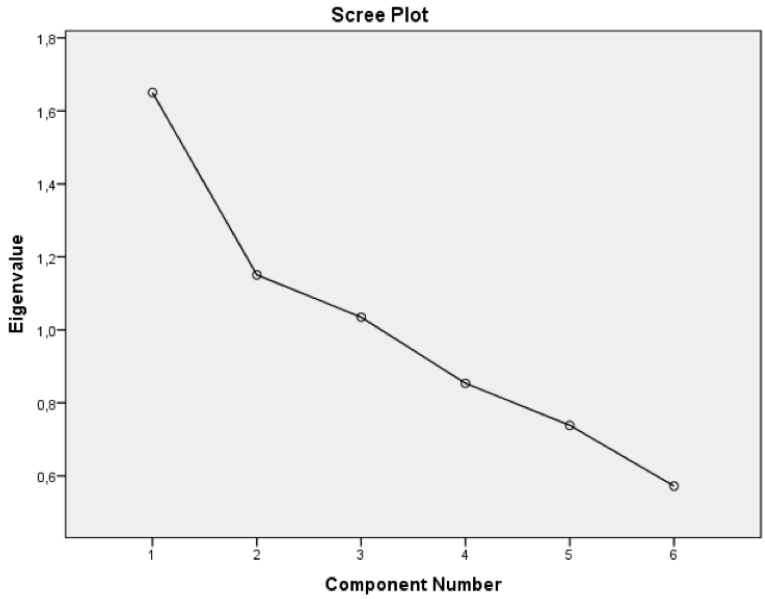


Figure 4.4: Principal component analysis - scree plot

Outcomes in bronchiolitis

Table 4.7 presents results on the relevance given by physicians to different outcomes. Mean scores for all outcomes were close to or above 4 in both groups, and all medians were 4 (interquartile range 3 to 5). Most outcomes were rated 4 or 5 by over 80% of participants in both groups. Ranking outcomes by highest overall mean score and by more frequent highest score led to comparable results on the top outcomes, which were hospital admission and respiratory distress for both pediatricians and GPs. Outcomes which less than 80% pediatricians scored 4 or 5 included feeding tolerance, treatment harms, return visits, quality of life and sleep; the latter three had the lowest mean scores of all outcomes. Scores for need for oxygen therapy, recurrent wheezing and asthma were more frequently less than 4 or 5 by GPs when compared to pediatricians, both being among the lowest mean scores in the GP group.

Table 4.7: Perspectives of paediatricians and general practitioners on relevance of outcomes in bronchiolitis

Outcome	Mean score (SD)	Scored 4/5 by >80%	% given outcome as highest score	Rank (by mean score/by % highest core)
PEDIATRICIANS (n=466)				
Hospital admission	4.38 (0.7)	yes	70	1/1
Length of hospital stay	4.29 (0.68)	yes	60.9	4/4
Return visits	3.99 (0.82)	no	44.2	10/9
Respiratory distress	4.37 (0.64)	yes	67.8	2/2
Need for oxygen therapy	4.36 (0.62)	yes	66.3	3/3
Feeding tolerance	4 (0.7)	no	40.8	9/11
Duration of illness	4.11 (0.74)	yes	50	6/8
Sleep[a]	3.83 (0.83)	no	35.6	12/12
Treatment harms	4.11 (0.79)	no	51.5	7/7
Quality of life	3.96 (0.81)	no	43.1	11/10
Pulmonary function	4.2 (0.78)	yes	59.2	5/5
Recurrent wheezing and asthma	4.1 (0.93)	yes	55.8	8/6
GENERAL PRACTITIONERS (n=119)				
Hospital admission	4.42 (0.8)	yes	74.8	2/2
Length of hospital stay	4.21 (0.85)	yes	59.7	5/5
Return visits	4.11 (0.94)	yes	53.8	9/10
Respiratory distress	4.5 (0.77)	yes	81.5	1/1
Need for oxygen therapy	3.99 (0.97)	no	50.4	12/11
Feeding tolerance	4 (0.87)	yes	47.1	11/12
Duration of illness	4.2 (0.84)	yes	58.8	6/8
Sleep[a]	4.18 (0.93)	yes	60.5	8/4
Treatment harms	4.19 (0.93)	yes	59.7	7/5
Quality of life	4.22 (0.89)	yes	59.7	4/5
Pulmonary function	4.33 (0.94)	yes	73.1	3/3
Recurrent wheezing and asthma	4.11 (0.97)	no	58.8	10/8

[a]Parent-reported measures
[b]Refers to long-term lung function prognosi

DISCUSSION

To our knowledge, this study provides the first comprehensive assessment of bronchiolitis definitions from physicians. The debate over the definition of disease has lasted for decades, as the same diagnostic label is applied to children with important differences in their demographic, history and physical examination features.[4,19,95,214,719,720,721] Distinct perspectives have often been attributed to a geographical divide between the North American definition favoring a first episode of wheeze in up to one- or two-year-olds, and the use of the term in the United Kingdom and Australia, with crackles/crepitations in younger infants as hallmarks, with or without wheeze.[4,721] However, empirical evidence in this field is scarce. Our results suggest pediatricians have a broader view on auscultatory findings, including both wheezing and crackles/rales. This is in line with the Nottingham consensus, as well as with definitions mentioned in current practice guidelines.[393,394] We did find considerable disagreement between pediatricians on age for acute bronchiolitis and number of episodes, and none of the guidance documents adheres to strict limits on these parameters. Further, results from GPs clearly show a different perspective for most items, with only wheezing as a common feature. Thus we confirmed that physicians, at both individual and specialty-level, define bronchiolitis differently and we have identified heterogeneous clinical items that must be addressed in any future standardized definition of bronchiolitis.

The heterogeneity in disease definition has considerable impact both for clinical practice and research in viral acute bronchiolitis. Variability between centers in diagnostic labeling of lower respiratory tract infections in young children has been shown to influence treatment practices, given the overlap with asthma and viral-induced wheeze.[55] We found a striking gap between physicians' perspectives and the definition used in recent large clinical trials, mostly due to its 12-month limit and restriction to a first episode. This has important implications for the external validity and implementation of trial findings. A majority of clinicians uses a broader definition of bronchiolitis that includes older children with recurrent episodes, and may find it hard to extrapolate results from these trials. On the other hand, they may not necessarily consider evidence from trials in children with recurrent pre-school wheezing as applicable to bronchiolitis. Conversely, there are arguments in favor of a strict definition.[725,802] Jartti et al showed both age and episodes are associated with factors such as viral etiology and atopic characteristics, which may influence

short- and long-term outcomes.[95] In clinical trials, randomization can balance these or other confounders that may affect treatment responses. However, both future trials and epidemiological, prognostic and even translational research in this field would benefit from a more robust case definition, with clear boundaries with other wheezing disorders. Since a balance is needed between study design and clinical practice, clarifying standardized definitions in current practice guidelines would help. Further, even if the clinical definition is kept broader, researchers could agree on major subgroups for stratification, based on current and upcoming markers for domains such as host susceptibility, agent virulence, immunopathogenesis and environmental predictors.

Our exploratory factor analysis added to these findings by identifying three key dimensions of correlated clinical features that underlie individual perspectives, what we may call 'physician-based phenotypes' of bronchiolitis. The weight given to each component varied by physician specialty and training, but not by practice or geographical location, hinting at differences due to clinical teaching and experience, or severity of disease seen across settings. These 'physician-based phenotypes' should be put against patient data to clarify whether valid clinical phenotypes of bronchiolitis exist. Interestingly, two dimensions match previously proposed clinical phenotypes based on auscultatory findings: one characterized by crackles (close to the 'coryza and crackles/rales, no sudden onset' component), the other by wheeze alone.[4] The acoustic characteristics and pathological correlates of adventitious sounds might be markers of distinct host responses in bronchiolitis, and results from one small study suggest they lead to different long-term respiratory outcomes.[734] Further, Sanchez et al found response to bronchodilator in infants with bronchiolitis could be predicted from wheeze characteristics.[622] However, validating such phenotypes requires more evidence on their association with disease severity, prognosis and possibly treatment response. Furthermore, while a separate 'age and episodes' component signals that physicians perceive these parameters independently, we've shown these and other putative phenotype-defining traits interact and must be approached coherently for an inclusive definition.[462]

Our study also addressed the critical issue of outcome selection, which has been often overlooked in bronchiolitis intervention research. Previous systematic reviews

of trials have repeatedly found variability in measured outcome domains and instruments used, noting the absence of guidance on clinically important outcomes (Chapters 2 and 3.1). Developing a core outcome set that is relevant to all stakeholders would be a major step forward in acute viral bronchiolitis research. We've made a first contribution by evaluating the physician perspective, and found hospital admission and respiratory distress as the highest ranked and rated outcomes. Interestingly, there was remarkable consistency between pediatricians and GPs for these top outcomes, but some differences were found in outcomes that scored below a commonly used threshold for consensus (i.e. 80%). Outcomes such as quality of life or sleep were more valued by GPs, which highlights the need to incorporate stakeholder perspectives across levels of care given the wide spectrum of severity of bronchiolitis. Overall, all listed outcomes were highly rated by either clinician group, and should be considered in a future core outcome set. Importantly, this study is just a preliminary step in a structured core outcome set development process.[682,691] Our results may be used to support a formal consensus approach involving key stakeholders (other clinicians, researchers, parents, regulators), across settings and internationally. Both bronchiolitis definition and outcome selection could be addressed in such an initiative.

Strengths and limitations of our study must be considered. First, we focused on a limited predefined set of clinical findings and outcomes, both of which may not encompass all relevant items. Our purpose was to generate representative data, and further in-depth analysis will require qualitative research techniques. Second, the demographics of participating pediatricians suggest they are likely representative nationwide and across settings, oversampled for residents. The sample of GPs was smaller and many worked in pediatric emergency; while primary care pediatrics is mainly provided by GPs, there could be a possible bias of our sample towards views closer to those of pediatricians. Moreover, answers in this survey may not accurately reflect individual practice when diagnosing bronchiolitis. Although we used standardized nomenclature of adventitious sounds, terminology varies and reliability of stethoscope examination is limited.[381] Lastly, there may be limits to the extrapolation of our nationwide results to other countries. Stakeholder perspectives on relevance of outcomes are likely to vary by factors such as organizational care and societal values, and may not be generalizable worldwide. On the other hand, Portugal has not been connoted to any of the "geographical" bronchiolitis definitions, which may be a strength of our study.

In summary, this survey showed pediatricians' and GPs' definitions of bronchiolitis are heterogeneous and often mismatch those of clinical trials. Exploratory component analysis identified domains that underlie different definitions. Our results highlight the need for a robust standardized definition that accommodates relevant subgroups and possibly phenotypes, suiting both clinical practice and research design. Further, we identified outcomes of most relevance to physicians, contributing to the development of a core outcome for future clinical trials.

CHAPTER 5

DISCUSSION, CONCLUSIONS AND FUTURE DIRECTIONS

In this thesis we have first provided a comprehensive and integrated systematic review with network meta-analysis on the comparative efficacy and safety of the two mostly frequently used groups of medications in bronchiolitis, i.e. corticosteroids and bronchodilators. Second, this body of evidence highlighted inconsistencies and limitations in outcome domains and measurement instruments reported in clinical trials with these interventions. We have further addressed the issue of outcome selection and measurement with a study on the measurement properties of the two most frequently used respiratory distress scales, RDAI and RACS. Finally, we have assessed physician perspectives on disease definition and important outcomes in bronchiolitis, as preliminary steps in obtaining a standardized definition and a core outcome set.

Here we summarize sequentially the main findings on the three topics addressed in this thesis (evidence on the use of corticosteroids and bronchodilators, outcome selection and measurement, and disease definition), and we discuss their implications for clinical practice and research, including directions for future studies.

Corticosteroids, bronchodilators and other interventions in bronchiolitis: next steps in evidence synthesis and trials

Main findings

Results from the comparative effectiveness review presented in **Chapter 2** provide greater clarity for clinical decision-making regarding the relative benefits and harms of corticosteroids and bronchodilators in bronchiolitis. Adrenaline was beneficial for short term outcomes among outpatients, including hospital admission rates from the ED on day one, while exploratory evidence suggested a longer term synergistic effect of combining this treatment with dexamethasone, with reduction in admissions up to seven days. Clinical scores and symptoms results supported this benefit. However, these were the findings of a single study with methodological issues, and should be interpreted cautiously. Further, exploratory subgroup analysis was not conclusive as to an additive/synergistic effect of corticosteroids combined with bronchodilators. While no relevant differences were found in short-term general and intervention-specific adverse effects for these interventions, harms of combined therapy need to be clarified further, including long-term safety data.

Importantly, current evidence does not support a clinically relevant stand-alone effect of systemic or inhaled corticosteroids, β2-AR agonist or anticholinergics on most measured outcomes. Also, none of the tested interventions were found to be beneficial in hospitalized patients. This is possibly because admitted patients may have failed prior treatments, and, in the hospital, were provided optimal supportive measures. Overall, both direct and indirect comparisons supported these findings, and mixed treatment comparisons allowed us to rank interventions with the highest probability of being most effective.

Implications for practice and research, future directions

For the design and conduct of future systematic reviews

One of the key strengths of this comparative effectiveness review is that uses consistent methods, including definition of disease and outcomes, set a priori at all levels of the review, including analytical methods, risk of bias and GRADE assessments.[662] This allowed us to overcome the methodological heterogeneity found in previous systematic reviews. Controversies still may occur, as results from these studies were disputed based on our exclusion of recurrent wheezers.[803] Individual participant data meta-analysis would allow to explore any differential treatment effects based on these individual factors.[804] Another strength was that network meta-analysis provided information on the relative effectiveness across a range of interventions, e.g. favoring adrenaline over other bronchodilators as a first choice for short term benefit.[805]

This approach sets a standard for future reviews in this field. It could be used to evaluate and incorporate new evidence on these interventions as it is being published, while expanding and comparing it to other treatments that are being increasing tested, such as nebulized hypertonic saline. Evidence from other domains, e.g. economical evaluations, could also be added. Methodology for network meta-analysis has been evolving quickly since we concluded our project, as has the number of published reviews using this type of analysis.[806] Developments include methods to evaluate the quality of evidence and risk of bias, to conceptualize and assess the assumptions underlying indirect and mixed treatment comparisons, to visualize treatment networks and to explore additive effects.[666,805,807-809] These methods will likely allow us to deal with the increasing complexity of evidence in bronchiolitis treatment, with many comparators, co-

interventions with different doses and modes of administration (e.g. normal or hypertonic saline), and supportive treatments (e.g. low-flow oxygen or high-flow nasal cannula), all of which may interact with synergistic or antagonistic effects. On the downside, comparative effectiveness reviews are resource intensive and time-consuming, thus less likely to be updated on a regular basis.[662] Within the timeframe of this thesis, we were able to update our Cochrane review on corticosteroids, but not the comprehensive review. Further, complex reviews and network analysis are not overly familiar or well understood by clinicians, and readers may be skeptical of indirect comparisons.[662] Many formats have been proposed to improve dissemination of evidence from systematic reviews to end users; results from our review contributed to a Continuous Medical Education activity (BMJ Learning module), an evidence synopsis (JAMA Clinical Evidence Synopsis), and a point of care online product (Cochrane Clinical Answer). While such comprehensive reviews may not always be feasible, homogenous inclusion criteria, outcomes and methods between individual systematic reviews would allow easier comparison between them. These reviews could be a starting point for identifying relevant primary studies and a source of study-level data for future network analysis, which could be conducted only when needed.[810]

<u>For the design and conduct of future clinical trials</u>

Our findings identify gaps in current evidence that may be addressed in future clinical trials. While adrenaline was favored for short-term benefit, results had some degree of imprecision and were sensitive to risk of bias, leaving room for further well-designed and adequately powered trials. The sample size for these trials could be calculated based on what is required for a conclusive and reliable meta-analysis, i.e. the required or optimal information size, using existing evidence from our review.[811] Issues of adrenaline dosing and dilution solution should be considered in such trial. Further, while guidelines often suggest a treatment trial to document clinical response to bronchodilator, this strategy has not been systematically tested and could be considered at the RCT design level, both for outpatients and inpatients.[394]

Combination therapy with corticosteroids and bronchodilators for outpatients is another obvious focus of interest for future trials, which may have different possible aims. First, replication is needed to confirm the robustness of our findings of benefit

from the association of adrenaline and high-dose dexamethasone. Second, while recent findings from a systematic review do not suggest any major short-term safety concern when using short-term courses of systemic corticosteroids in children with acute respiratory conditions, future trials should contemplate comprehensive short and long term safety data.[641] Finally, studies should identify the minimum efficacious dose and type of corticosteroid, and whether other bronchodilators are also beneficial.

One of the many challenges in designing and conducting clinical trials in bronchiolitis is the choice of comparators. The wide and persistent variability in use of bronchodilators makes it harder to achieve consensus when choosing acceptable comparators for multicenter protocols. This may also be an obstacle when obtaining funding or implementing results. Preference for active comparators is problematic, as most interventions have not been proven to be truly effective when compared to placebo. Conversely, placebo arms often consist of nebulized saline or similar solutions, and both the solution and the mode of administration per se can induce a beneficial or harmful "placebo" effect.[812] For example, Skjerven et al recently showed that an "on-demand" strategy was superior over "fixed-schedule" use, regardless of the nebulized treatment, hinting at a harmful effect of nebulization in inpatients.[813] Design of future trials must consider these findings, and our results identify candidate comparators for future trials, while future network analyses may use integrate indirect evidence for all comparisons.

Evidence is emerging about promising treatments for bronchiolitis patients, either existing interventions such as hypertonic saline and high-flow oxygen, or new antiviral drugs or biologics.[786,814] As these new treatments are being tested or used, it is important to highlight how long-lasting claims of efficacy of many decade-old and widely used interventions are challenged by a growing evidence base such as that from this review. Thus, it is paramount that these treatments are thoroughly assessed for their efficacy and safety in well-design, adequately powered trials, in order to support their possible role in the management of bronchiolitis. Further, as discussed below, it is important use standardized definitions and to consider subgroups of interest a priori, in order to explore any differential treatment effects, and in view of future data sharing and meta-analysis.

Towards a core outcome set of domains and measurement tools in bronchiolitis

Main findings

One of the key limitations of our comparative effectiveness review was the heterogeneity in the selection of outcomes and outcome measurements in included bronchiolitis trials. **Chapter 3** shows how reported outcome measurements were mostly restricted to short-term clinician-based clinical severity/respiratory distress and healthcare use domains, while few measured caregiver-reported symptoms and quality of life, or long-term outcomes. The same was found for outcomes used to power these trials. Further, more than 20 different measurement instruments were identified, with different timings of measurement, metrics and methods of analysis.

Results presented in **Chapter 4** provide a first contribution to assess physician perspectives on relevant outcomes and outcome domains in bronchiolitis trials. Given the scarcity of quantitative and qualitative evidence in this field, the purpose of this project was to generate preliminary large scale representative data from physicians working in different settings and specialties relevant to bronchiolitis management. The top ranked and rated outcomes by both pediatricians and GPs were hospital admission and respiratory distress. Most outcomes that pediatricians scored above a commonly used threshold for consensus (i.e. 80%) were focused on core areas and domains of health resource use (hospital admission and length of stay), and pathophysiological manifestations, including clinical severity (respiratory distress and need for oxygen therapy), pulmonary function, and disease-related long-term manifestations (recurrent wheezing and asthma). Outcomes relating to life impact, such as quality of life or sleep were more valued by GPs.

Physicians rated respiratory distress as a key outcome domain for bronchiolitis trials, and this domain was often measured in clinical trials of corticosteroids and bronchodilators using the RDAI and RACS. In **Chapter 3.2**, we provide data on the validity, reliability and responsiveness of these scales. We found that RDAI had limited validity according to our predefined physiological and decision-making constructs, as it did not meet all our hypothesis. Inter-rater and test-retest reliability were good, showing RDAI has adequate discriminative properties. There was considerable test-retest measurement error which is a limitation, particularly for its longitudinal use at an individual level in clinical practice. Both RDAI and RACS

were moderately responsive according to our hypotheses, with RACS being slightly more responsive. These results suggest that both scales may be suitable for use as evaluative trial outcome measures, with attention needed to measurement error.

Implications for practice and research, future directions

For the development of a core domain set for future trials in bronchiolitis

The preliminary work on reviewing reported outcomes suggests there are gaps in measured outcome domains and discrepancies in measurement instruments in bronchiolitis trials. However, evidence from our sample of trials may not reflect all domains and instruments used to date in this field. For example, our sources of data did not include trials for all existing interventions and in all settings. Given the spectrum of disease severity in bronchiolitis, as well as the wide range of existing interventions, including both drug, device and supportive treatments, it is likely that reported measured outcome domains and instruments vary. Further, we focused on RCTs, but observational prognostic studies may add information on relevant outcome domains (e.g. harms, symptoms-related, quality of life). Importantly, they may report the use of other evaluative instruments of interest, and include other timings of measurement. Finally, we used a restrictive definition of bronchiolitis by excluding trials with participants with a history of wheezing; these trials may also contribute relevant information regarding outcomes. A next step would be to broaden the literature search and widen the inclusion criteria, in order to have a comprehensive on currently measured outcome domains and chosen instruments. Further, as new treatments for bronchiolitis are emerging recently, some of which at early phases of clinical development plans under regulatory supervision, it would be interesting to scope which outcomes domains and instruments are being selected for these ongoing and registered trials.

Further in-depth analysis of physician and other health care practitioners and clinical researchers' perspectives is needed through qualitative research techniques. This would allow for the inclusion of additional items, and to explore issues such as feasibility of outcome measurement (e.g. in the case of lung function testing) and timings of measurement. It is essential to consult other stakeholders groups, particularly parents and caregivers of children with bronchiolitis, to determine what they deem essential to measure. Results from a more comprehensive literature review and explicit input from all stakeholders would be incorporated in a

conceptual framework of outcome domains, and formal consensus techniques could be used to achieve consensus on important outcome domains. Methodological aspects to consider in this process include: the impact in parent and clinician perspectives of factors such as disease severity, settings, specialties, different types of interventions and individual patient characteristics (e.g. age, comorbidities, recurrent wheezing); and attention to both short- and long-term effects of bronchiolitis, despite uncertainty on the latter. Any further steps should take into account ongoing developments in the methodology for core outcome set development, including conceptual frameworks, quality assessment instruments, and effective methods for engaging, informing and obtaining consensus among key stakeholder groups in an iterative process that should be updated.[682,688,689,692] Study protocols in which key decisions are documented regarding the choices made in the process of core outcome set development are emerging. Further, future implementation of core outcome sets should be considered upfront. For example, while the consistency of measurement of OMERACT's core set of outcomes for rheumatoid arthritis has improved since the introduction, variation in the choice of measurement instrument remains.[815] This should be considered with care in bronchiolitis, which involves stakeholders with different perspectives across settings and with wide practice variation.

<u>For the development of a core outcome set of adequate measurement instruments in bronchiolitis</u>

The aforementioned comprehensive literature review could identify existing instruments for each domain, and candidate instruments would then be evaluated by systematic reviews of studies assessing their measurement properties or other quality and applicability filters. An inclusive approach is needed to ensure that all domains are covered, and matching adequate instruments are found. Further, gaps in available instruments can be identified and lead to the development of new tools or adjustments in current ones. For some relevant domains, it is likely that instruments exist that may be not have been developed and tested specifically in bronchiolitis, but in related conditions (e.g. wheezing disorders) are still useful, e.g. regarding respiratory symptoms, quality of life. Thus it is important to have a broadened view on candidate instruments, based on expert content in each domain. It is also relevant to note that the COMET and COSMIN initiatives are working on guidelines to support outcome measurement instrument selection (Core

Outcome Measurement Instrument Selection project - COMIS), and OMERACT is reviewing its filter and guidance on the procedures to document applicability of instruments.[691,816] Such developments should be included in a comprehensive effort to select measurement instruments for outcome domains in bronchiolitis.

RDAI does not encompass all dimensions of the respiratory domain, nor of other domains of disease manifestations and severity of bronchiolitis. RDAI was initially proposed as a respiratory scale focusing on variables that reflected underlying wheezing pathophysiology and that were frequently used in clinical practice. However, the scale was developed ad hoc with no elaboration on the underlying conceptual model and construct to be measured, nor any rationale for item selection, scoring method or empirical weighting. Few respiratory distress and wheezing/asthma scales have been developed or validated in bronchiolitis.[694] Despite the overlap between conditions, it is likely that differences in clinical findings, pathophysiology and course of the disease influence the properties of measurement instruments. RDAI and RACS measurement properties should now be compared with those of other scales that have been tested in adequate measurement studies. This will highlight strengths and limitations of current instruments for outcome measurement of this domain. A consensus approach to instrument selection based on this body of evidence would allow to choose what is the most adequate and feasible instrument. It will also help identify whether new instruments focused on this domain or encompassing also other domains are needed. Few instruments in this field have been developed using a structured approach. A recent project is developing one such bronchiolitis severity scoring instrument for use by nurses and other healthcare professions.[817]

<u>For further study of RDAI and RACS</u>

Another open question regarding RDAI and RACS refers to the interpretability of change scores. Ascertaining the MIC of these scales would be useful when designing and interpreting studies, e.g. to define the proportion of responders.[818] Authors have also proposed that the MIC (or MID, depending on terminology) could be used to facilitate the interpretation of pooled results from clinical scores in meta-analysis.[819] However, methodology is still evolving as to which methods are most appropriate to obtain the MIC, or a range of MICs.[671] The MIC depends on a number of factors, including choice of anchors, who assesses important change,

time interval during which change is being assessed, and whether improvement and/or deterioration are considered. Given the limitations of our data set, particularly the absence of a formal patient or physician anchor of change, these issues must be explored carefully before suggesting a "magical number" as an MIC.

Back to basics: standardizing bronchiolitis definitions

Main findings

Chapter 4 addressed another key shortcoming in current intervention research, i.e. the absence of a consistent definition of bronchiolitis. Results form our nationwide survey found that there is variability at both individual and specialty-level in how physicians define bronchiolitis, and these definitions often mismatch those of clinical trials. We identified heterogeneous clinical items that must be addressed in any future standardized definition of bronchiolitis, particularly age, number of episodes and auscultatory findings. Further, exploratory factor analysis identified key dimensions of correlated clinical features that underlie individual perspectives, which we called 'physician-based phenotypes' of bronchiolitis: one based on 'coryza and crackles/rales, no sudden onset', another on 'age and episodes', and a last one on 'wheeze'.

Implications for practice and research, future directions

<u>For the the development of a standardized definition of bronchiolitis</u>

Most research in bronchiolitis would benefit from a standardized, consistent definition of disease. This includes clinical trials, prognostic studies and translational studies that would use such definition as part of their inclusion criteria, but also epidemiological studies using bronchiolitis as an outcome measure. In turn, it would allow consistency and comparability in systematic reviews of these studies. One such definition would also have to strike a balance with the pragmatic implications for clinical practice, e.g. regarding disease labeling and management options. A key challenge is to obtain an operational definition that is consistent but allows for different traits and phenotypes; rigorous and validated e.g. regarding pathophysiology, severity and prognosis; and likely to be accepted by different stakeholders.

In this regard it is useful to reflect on two different perspectives on disease definition and classification of airway disorders proposed by Wardlaw and colleagues, that of 'lumpers' versus that of 'splitters'.[736] The former are said to prefer to recognize commonality in disease processes, but risk denying the insight that accurate classification brings to understanding the disease. For example, the diagnostic term 'asthma' was originally coined to categorize a clinical presentation that appeared to comprise a more or less distinct disease process. As medical science progressed and our knowledge of measurable physiological, pathological and molecular abnormalities associated with disease processes became known, attempts to match these abnormalities to the original disease classification have been problematic. Thus in later years, the use of this term without any qualification or definition has begun to hinder rather than facilitate progress in research.[820] On the contrary, there are those who emphasize complexity in disease definition, but can end up with endless and useless subgroups if classification is not grounded in pathogenesis. The phenotype and endotype approach we discussed in the introduction can carry that risk.

A balance between both approaches could consist in deciding on an inclusive definition and agreeing on major subgroups for stratification. For example, using our results, most pediatricians agreed with the Nottingham guideline definition, in children up to 24 months of age and with up to three episodes. However, as mentioned in the Introduction, including recurrent wheezers and older children is problematic, as these parameters may be proxy for asthma-prone children with distinct viral susceptibilities, preexisting inflammation and/or lung function. Conversely, a restricted definition might help obtain a more homogenous population, at the expense of being possibly less accepted in practice, and leaving some children in "limbo" of uncertain definition e.g. older children with a second or third episode. In any case, subgroups could be defined based on different putative phenotypes related to physician perspectives (as we identified them), clinical and host susceptibility parameters (e.g. restricted to children below 6 months, or to children with wheezing), agent virulence (e.g. RSV vs RV), environmental predictors (e.g. history of atopy), or any upcoming immunopathogenesis biomarkers. These subgroups could be validated based on underlying disease mechanisms, and their association with disease severity and prognosis. They could also be used for stratification to assess differential treatment

response in trials. In any case, a clear distinction should be made on the implications for research and for practice of any standardized definition and different subgroups.

From the above, it ensues that a formal consensus procedure involving representative stakeholders is needed to obtain one such disease definition. Key stakeholders could include health care practitioners, researchers of different fields, and guideline developers. Care should be taken to account for the regional and international variation in bronchiolitis definition. Existing initiatives have focused on disease definition using consensus procedures, e.g. the ROME conference for functional gastrointestinal disorders.[821] Both bronchiolitis definition and core outcome set development could be addressed in parallel and collaborative initiatives, given the similarity of consensus approaches and how both issues are related.

ACKNOWLEDGEMENTS

Related to specific projects

I thank the following collaborators for their contributions to the systematic review projects presented in Chapter 2: Annabritt Chisholm (article retrieval), Andrea Milne and Heather McPhee (data extraction), Nicola Hooton (protocol development, study selection), Ozge Tunçalp (Turkish translation), Joy Lee (Korean translation), Paolo Valerio (Dutch translation), and João Franco (Spanish translation). I also thank the Cochrane Collaboration Acute Respiratory Infections Group editorial team, particularly Liz Dooley (Managing Editor) and Sarah Thorning (Trials Search Coordinator), for supporting the updated version of the Cochrane review. Projects in Chapter 2 were supported by the Canadian Institutes of Health Research knowledge synthesis grant program, and by the Cochrane Incentives Scheme Funding for Cochrane Systematic Reviews from the UK Cochrane Center.

Results presented in Chapter 3.2 would not have been possible without the contribution of all the children and families who participated in CanBEST and the prognostic cohort study. I thank all site research coordinators, coordinating site staff, research nurses and participating site physicians and nurses, and all Pediatric Emergency Research Canada members who were involved in the design, conduct and reporting of both multi-centre studies. My special thanks to Nicola Hooton (data manager) for her help with database queries. CanBEST was supported by grants from the Canadian Institutes of Health Research and Alberta Children's Hospital Foundation.

The electronic survey presented in Chapter 4 was developed with funding and support from the Pulmonology Section of the Portuguese Society of Pediatrics (SPP) and the Portuguese Association of General Practitioners (APMGF). I would like to acknowledge Pedro Mendes (Portuguese Society of Pediatrics) for managing the electronic contact database. I thank all team members involved for their fruitful collaboration (in particular Carolina Constant for helping design the survey, Daisy Abreu for statistical support and Maria Gabriela Andrade for hands-on work on this project), as well as all physicians that participated in the survey nationwide.

For all projects, I was supported by the Program for Advanced Medical Education, sponsored by Fundação Calouste Gulbenkian, Fundação Champalimaud, and Ministério da Saúde e Fundação para a Ciência e Tecnologia, Portugal.

General acknowledgements

To everyone at the former Pediatric Clinical Epidemiology unit at the Academic Medical Centre in Amsterdam, where much of this journey started, thank you for the sunny memories despite the everlasting dutch rain. To Martin Offringa, now at the centre stage of child health research at SickKids in Toronto, for his sharp guidance and mentorship throughout the years, for teaching me through action and for sharing his culture and insight, in short, for his decisive contribution to my career as a clinical researcher and for the many opportunities I was given along this journey. To Hanneke van der Lee, without whom this thesis would have hardly gone forward, for her close guidance in both epidemiology and the dutch way - she is the one to blame for how measurement science somehow found a way to quietly creep into me! Close regards also to Marjan du Prie and more recently to Martine Jansen-van der Weide, who accompanied this long path at different crossroads. A word of thanks to Caroline Terwee, at VU University in Amsterdam, always at the forefront of measurement research, for the honor of having her late coming but decisive contribution to the measurement study. A final word to my friends Nuno Marcelino and Patrícia Almeida, who always welcomed me warmly into their home in Amsterdam.

To all the team at the Alberta Research Centre for Health Evidence in Edmonton, and other collaborators in Canada, a warm (pun intended) thank you for your reception and continued support. It's funny how overseas seems next door when we share so much common ground. In particular, to Liza Bialy, for her commitment to the systematic review projects, to Ben Vandermeer, for his patience and no-nonsense approach when dealing with all my overenthusiastic and naive statistical inquiries, and to Amy Plint and all PERC investigators, whose landmark CanBEST project was the backbone of this thesis, for the generosity of allowing me to work freely with this data despite my fussiness about the almost-polar weather (my Perky prize will be remembered!). Last but not least, to Terry Klassen, for his wisdom and vision, which was just as important for me as his pivotal research work I had come to admire before knowing him, and to the mover and shaker Lisa Hartling, for her

immense energy and determination, and for the pleasure and honor to continue working together in child health research methodology.

To all members of the StaR Child Health initiative, in particular members of Standard Development Groups on Data Monitoring Committees, Adequate Sample Sizes, and Selection, Measurement, and Reporting of Outcomes, for the experience of contributing to a remarkable high-quality international initiative that is not without its challenges - many lessons learned indirectly made it into this thesis. To members of the COMET initiative, in particular to Paula Williamson at the University of Liverpool, for providing me with an opportunity to present my work and for her vision and commitment to the issue of outcomes. A final word to Ian Sinha in Liverpool, for his groundbreaking work on outcomes in pediatrics and asthma, that was an inspiration for some of my projects and had the unintended outcome of a lasting friendship.

To the Gulbenkian Program for Advanced Medical Education, in particular to Leonor Parreira for her vision, courage and support, and for being a leading example of rigor and commitment in clinical research, to António Coutinho for sharing his limitless culture, wisdom and passion for science, and to João Ferreira for being a reassuring brotherly arm full of scientific wisdom. To all my colleagues at the Program, in particular those that I had the privilege to share hopes and doubts with during the first edition - Nuno, Ana(s), Joana, Filipe, Sandra, Maria Rita, Sofia, Teresa - this has been a long and often challenging journey, may we be the change that is so much needed. In memoriam, Jaime Almeida.

To everyone at the Department of Pediatrics, Hospital de Santa Maria, including all my Directors, in particular João Gomes-Pedro, for being decisive in supporting my submission to the doctoral program, then later Paulo Ramalho and now Maria do Céu Machado, both of whom continued to actively back my involvement in clinical research. To all my colleagues at the Pediatric Pulmonology Unit, Luisa Pereira, Rosário Ferreira, Teresa Nunes, Celeste Barreto, Ana Saianda, Carolina Constant, Elsa Santos, for always supporting my endeavors even as there seemed to be no end in sight, and for sharply bringing me down to earth, so that I never forget that at the end of the day clinical research is about improving patient health. To my colleagues at the Pediatric Allergy Unit, Ana Neves and Joana Fermeiro, for their support. To

everyone working rounds at Pediatric Emergency, consultants, residents and students, may we manage each season of bronchiolitis with the right balance of evidence and experience. A heartfelt word to José Costa Trindade, for his patience in accompanying this endless journey, and for sharing his invaluable experience and advice at key moments. Finally, to Teresa Bandeira, my clinical mentor and friend, thank you for your leadership, vision and guidance that so often pointed the right direction amidst the foggy road.

To everyone at the Laboratory of Clinical Pharmacology and Therapeutics / Clinical Pharmacology Unit, at the Faculty of Medicine, University of Lisbon, and Instituto de Medicina Molecular, in particular to Cristina Sampaio, for giving me her full support as I entered the brave new world of methodology and clinical pharmacology, for her critical advice at a distance and for providing me with a safe haven for my clinical research work. To Joaquim Ferreira, for his mentorship, friendship and trust, and for teaching me to aim high and not compromise scientific rigor and ethics as we navigate the troubled waters of clinical research in our setting. Finally, to all other colleagues and staff, for helping me with details and trivia that are so often key to getting you through the day.

A warm thanks to Inês Pargana (aka BellySketcher) for her beautiful work on this thesis cover.

To my parents and my sister, who taught me the ethics of work, humanity and love.

This thesis is dedicated to Raquel and Henrique, my motives of faith and meaning, my precious star-stuff navigating the magnificence of cosmic indifference.

REFERENCES

1. Wright AL, Taussig LM, Ray CG, Harrison HR, Holberg CJ, The Group Health Medical A. The Tucson Children's Respiratory Study: II. Lower Respiratory Tract Illness In The First Year Of Life. Am J Epidemiol 1989; 129(6): 1232-46.

2. Smyth RL, Openshaw PJ. Bronchiolitis. Lancet 2006; 368(9532): 312-22.

3. Bush A, Thomson AH. Acute bronchiolitis. BMJ 2007; 335(7628): 1037-41.

4. Everard ML. Acute Bronchiolitis and Croup. Pediatr Clin North Am 2009; 56(1): 119-33.

5. Mansbach JM, Piedra PA, Teach SJ, et al. Prospective multicenter study of viral etiology and hospital length of stay in children with severe bronchiolitis. Arch Pediatr Adolesc Med 2012; 166(8): 700-6.

6. Collins PL, Graham BS. Viral and host factors in human respiratory syncytial virus pathogenesis. J Virol 2008; 82(5): 2040-55.

7. Sly PD, Kusel M, Holt PG. Do early-life viral infections cause asthma? J Allergy Clin Immunol 2010; 125(6): 1202-5.

8. Plint AC, Johnson DW, Wiebe N, et al. Practice Variation among Pediatric Emergency Departments in the Treatment of Bronchiolitis. Acad Emerg Med 2004; 11(4): 353-60.

9. Babl FE, Sheriff N, Neutze J, Borland M, Oakley E. Bronchiolitis management in pediatric emergency departments in Australia and New Zealand: a PREDICT study. Pediatr Emerg Care 2008; 24(10): 656-8.

10. Bialy L, Foisy M, Smith M, Fernandes RM. The Cochrane Library and the Treatment of Bronchiolitis in Children: An Overview of Reviews. Evid Based Child Health 2011; 6(1): 258-75.

11. Spender JK. Some Points in the Pathology and Treatment of Acute Capillary Bronchitis. BMJ 1868; 2(403): 304-5.

12. Clark CP, Batman FH. Pneumococcal Bronchiolitis (Capillary Bronchitis). J Infect Dis 1904; 1(2): 229-35.

13. Engel, Newns GH. Proliferative Mural Bronchiolitis. Arch Dis Child 1940; 15(84): 219-29.

14. Newns GH. Bronchiolitis in Children. Proc R Soc Med 1944; **37**(10): 580-5.

15. Hubble D, Osborn GR. Acute Bronchiolitis in Children. Br Med J 1941; **1**(4177): 107-26.1.

16. Adams JM. Primary virus pneumonitis with cytoplasmic inclusion bodies: Study of an epidemic involving thirty-two infants, with nine deaths. J Am Med Assoc 1941; 116(10): 925-33.

17. Heycock JB, Noble TC. An epidemic of acute bronchiolitis in infancy. Br Med J 1956; 1(4964): 438-9.

18. Disney ME, Sandiford BR, Cragg J, Wolff J. Epidemic bronchiolitis in infants. Br Med J 1960; 1(5183): 1407-11.

19. Winter ST. Bronchiolitis—What's in a Name? Clin Pediatr (Phila) 1971; 10(9): 485.

20. Chanock R, Finberg L. Recovery From Infants With Respiratory Illness Of A Virus Related To Chimpanzee Coryza Agent (Cca): Epidemiologic Aspects Of Infection In Infants And Young Children. Am J Epidemiol 1957; 66(3): 291-300.

21. Chanock R, Roizman B, Myers R. Recovery From Infants With Respiratory Illness Of A Virus Related To Chimpanzee Coryza Agent (Cca): Isolation, Properties And Characterization. Am J Epidemiol 1957; 66(3): 281-90.

22. Morris JA, Blount RE, Savage RE. Recovery of Cytopathogenic Agent from Chimpanzees with Coryza. Exp Biol Med 1956; 92(3): 544-9.

23. Beem M, Wright FH, Hamre D, Egerer R, Oehme M. Association of the Chimpanzee Coryza Agent with Acute Respiratory Disease in Children. N Engl J Med 1960; 263(11): 523-30.

24. Adams JM, Imagawa DT, Zike K. Epidemic bronchiolitis and pneumonitis related to respiratory syncytial virus. J Am Med Assoc 1961; 176: 1037-9.

25. Forbes JA, Bennett NM, Gray NJ. Epidemic bronchiolitis caused by a respiratory syncytial virus: clinical aspects. Med J Aust 1961; 48(2): 933-5.

26. Parrott RH, Vargosko AJ, Kim HW, et al. Respiratory syncytial virus. II. Serologic studies over a 34-month period of children with bronchiolitis, pneumonia, and minor respiratory diseases. J Am Med Assoc 1961; 176: 653-7.

27. Dabbous IA, Tkachyk JS, Stamm SJ. A double blind study on the effects of corticosteroids in the treatment of bronchiolitis. Pediatrics 1966; 37(3): 477-84.

28. Phelan PD, Williams HE. Sympathomimetic Drugs In Acute Viral Bronchiolitis: Their Effect on Pulmonary Resistance. Pediatrics 1969; 44(4): 493-7.

29. Kim HW, Canchola JG, Brandt CD, et al. Respiratory Syncytial Virus Disease In Infants Despite Prior Administration Of Antigenic Inactivated Vaccine. Am J Epidemiol 1969; 89(4): 422-34.

30. Reynolds EO, Cook CD. The Treatment Of Bronchiolitis. J Pediatr 1963; 63: 1205-7.

31. Sommer C, Resch B, Simões EA. Risk factors for severe respiratory syncytial virus lower respiratory tract infection. Open Microbiol J 2011; 5: 144-54.

32. Mejias A, Dimo B, Suarez NM, et al. Whole Blood Gene Expression Profiles to Assess Pathogenesis and Disease Severity in Infants with Respiratory Syncytial Virus Infection. PLoS Med 2013; 10(11): e1001549.

33. Marasco WA, Sui J. The growth and potential of human antiviral monoclonal antibody therapeutics. Nat Biotechnol 2007; 25(12): 1421-34.

34. Blanco JC, Boukhvalova MS, Shirey KA, Prince GA, Vogel SN. New insights for development of a safe and protective RSV vaccine. Hum Vaccin 2010; 6(6): 482-92.

35. Merkel OM, Rubinstein I, Kissel T. siRNA Delivery to the lung: What's new? Adv Drug Deliv Rev 2014.

36. Ralston SL, Lieberthal AS, Meissner HC, et al. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. Pediatrics 2014; 134(5): e1474-502.

37. Shay DK, Holman RC, Newman RD, Liu LL, Stout JW, Anderson LJ. Bronchiolitis-associated hospitalizations among US children, 1980-1996. J Am Med Assoc 1999; 282(15): 1440-6.

38. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet 2010; 375(9725): 1545-55.

39. Fouzas S, Priftis KN, Anthracopoulos MB. Pulse Oximetry in Pediatric Practice. Pediatrics 2011; 128(4): 740-52.

40. Ganu SS, Gautam A, Wilkins B, Egan J. Increase in use of non-invasive ventilation for infants with severe bronchiolitis is associated with decline in intubation rates over a decade. Intensive Care Med 2012; 38(7): 1177-83.

41. Zhou H, Thompson WW, Viboud CG, et al. Hospitalizations associated with influenza and respiratory syncytial virus in the United States, 1993-2008. Clin Infect Dis 2012; 54(10): 1427-36.

42. Dawson KP, Long A, Kennedy J, Mogridge N. The chest radiograph in acute bronchiolitis. J Paediatr Child Health 1990; 26(4): 209-11.

43. Perlstein PH, Kotagal UR, Bolling C, et al. Evaluation of an Evidence-based Guideline for Bronchiolitis. Pediatrics 1999; 104(6): 1334-41.

44. Barben J, Hammer J. Current management of acute bronchiolitis in Switzerland. Swiss Med Wkly 2003; 133(1-2): 9-15.

45. Corneli HM, Zorc JJ, Mahajan P, et al. A multicenter, randomized, controlled trial of dexamethasone for bronchiolitis. N Engl J Med 2007; 357(4): 331-9.

46. Plint AC, Johnson DW, Patel H, et al. Epinephrine and dexamethasone in children with bronchiolitis. N Engl J Med 2009; 360(20): 2079-89.

47. Koehoorn M, Karr CJ, Demers PA, Lencar C, Tamburic L, Brauer M. Descriptive epidemiological features of bronchiolitis in a population-based cohort. Pediatrics 2008; 122(6): 1196-203.

48. Pérez-Yarza EG, Moreno A, Lázaro P, Mejías A, Ramilo O. The association between respiratory syncytial virus infection and the development of childhood asthma: a systematic review of the literature. Pediatr Infect Dis J 2007; 26(8): 733-9.

49. Thompson M, Vodicka TA, Blair PS, et al. Duration of symptoms of respiratory tract infections in children: systematic review. BMJ 2013; 347: f7027.

50. Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ, Martinez FD. Tucson Children's Respiratory Study: 1980 to present. J Allergy Clin Immunol 2003; 111(4): 661-75.

51. Keil T, Kulig M, Simpson A, et al. European birth cohort studies on asthma and atopic diseases: II. Comparison of outcomes and exposures – a GA2LEN initiative. Allergy 2006; 61(9): 1104-11.

52. Turner S. Childhood respiratory cohort studies: do they generate useful outcomes? Breathe 2012; 8(3): 194-204.

53. Carroll KN, Gebretsadik T, Griffin MR, et al. Increasing Burden and Risk Factors for Bronchiolitis-Related Medical Visits in Infants Enrolled in a State Health Care Insurance Plan. Pediatrics 2008; 122(1): 58-64.

54. Hall CB, Weinberg GA, Iwane MK, et al. The Burden of Respiratory Syncytial Virus Infection in Young Children. N Engl J Med 2009; 360(6): 588-98.

55. Mansbach JM, Espinola JA, Macias CG, Ruhlen ME, Sullivan AF, Camargo CA. Variability in the Diagnostic Labeling of Nonbacterial Lower Respiratory Tract Infections: A Multicenter Study of Children Who Presented to the Emergency Department. Pediatrics 2009; 123(4): e573-e81.

56. Glezen W, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. Am J Dis Child 1986; 140(6): 543-6.

57. Houben ML, Bont L, Wilbrink B, et al. Clinical Prediction Rule for RSV Bronchiolitis in Healthy Newborns: Prognostic Birth Cohort Study. Pediatrics 2011; 127(1): 35-41.

58. Panickar JR, Dodd SR, Smyth RL, Couriel JM. Trends in deaths from respiratory illness in children in England and Wales from 1968 to 2000. Thorax 2005; 60(12): 1035-8.

59. Che D, Nicolau J, Bergounioux J, Perez T, Bitar D. Bronchiolite aiguë du nourrisson en France : bilan des cas hospitalisés en 2009 et facteurs de létalité. Arch Pediatr 2012; 19(7): 700-6.

60. Hasegawa K, Tsugawa Y, Brown DFM, Mansbach JM, Camargo CA. Trends in Bronchiolitis Hospitalizations in the United States, 2000–2009. Pediatrics 2013; 132(1): 28-36.

61. Fleming DM, Pannell RS, Cross KW. Mortality in children from influenza and respiratory syncytial virus. J Epidemiol Community Health 2005; 59(7): 586-90.

62. Shay DK, Holman RC, Roosevelt GE, Clarke MJ, Anderson LJ. Bronchiolitis-Associated Mortality and Estimates of Respiratory Syncytial Virus—Associated Deaths among US Children, 1979–1997. J Infect Dis 2001; 183(1): 16-22.

63. Yorita KL, Holman RC, Sejvar JJ, Steiner CA, Schonberger LB. Infectious Disease Hospitalizations Among Infants in the United States. Pediatrics 2008; 121(2): 244-52.

64. Langley JM, LeBlanc JC, Smith B, Wang EEL. Increasing Incidence of Hospitalization for Bronchiolitis among Canadian Children, 1980–2000. J Infect Dis 2003; 188(11): 1764-7.

65. Murray J, Bottle A, Sharland M, et al. Risk Factors for Hospital Admission with RSV Bronchiolitis in England: A Population-Based Birth Cohort Study. PLoS ONE 2014; 9(2): e89186.

66. Bjor O, Braback L. A retrospective population based trend analysis on hospital admissions for lower respiratory illness among Swedish children from 1987 to 2000. BMC Public Health 2003; 3: 22.

67. van Woensel JBM, van Aalderen WMC, Kneyber MCJ, Heijnen MLA, Kimpen JLL. Bronchiolitis hospitalisations in the Netherlands from 1991 to 1999. Arch Dis Child 2002; 86(5): 370-1.

68. Mansbach JM, Piedra PA, Stevenson MD, et al. Prospective Multicenter Study of Children With Bronchiolitis Requiring Mechanical Ventilation. Pediatrics 2012; 130(3): e492-e500.

69. Paramore LC, Ciuryla V, Ciesla G, Liu L. Economic impact of respiratory syncytial virus-related illness in the US: an analysis of national databases. Pharmacoeconomics 2004; 22(5): 275-84.

70. Pelletier AJ, Mansbach JM, Camargo CA. Direct Medical Costs of Bronchiolitis Hospitalizations in the United States. Pediatrics 2006; 118(6): 2418-23.

71. Hall CB. Nosocomial Respiratory Syncytial Virus Infections: The "Cold War" Has Not Ended. Clin Infect Dis 2000; 31(2): 590-6.

72. Simon A, Khurana K, Wilkesmann A, et al. Nosocomial respiratory syncytial virus infection: impact of prospective surveillance and targeted infection control. Int J Hyg Environ Health 2006; 209(4): 317-24.

73. Langley JM, LeBlanc JC, Wang EEL, et al. Nosocomial Respiratory Syncytial Virus Infection in Canadian Pediatric Hospitals: A Pediatric Investigators Collaborative Network on Infections in Canada Study. Pediatrics 1997; 100(6): 943-6.

74. Groothuis J, Bauman J, Malinoski F, Eggleston M. Strategies for prevention of RSV nosocomial infection. J Perinatol 2008; 28(5): 319-23.

75. Fournel I, Soulias M, Bour JB, Gouyon JB, Huet F, Aho LS. Évolution du nombre d'infections à Rotavirus et à virus respiratoire syncytial chez les enfants hospitalisés au CHU de Dijon entre 1998 et 2005. Pathol Biol (Paris) 2010; 58(6): 406-14.

76. Weedon KM, Rupp AH, Heffron AC, et al. The impact of infection control upon hospital-acquired influenza and respiratory syncytial virus. Scand J Infect Dis 2013; 45(4): 297-303.

77. Simon A, Ammann R, Wilkesmann A, et al. Respiratory syncytial virus infection in 406 hospitalized premature infants: results from a prospective German multicentre database. Eur J Pediatr 2007; 166(12): 1273-83.

78. Thorburn K, Eisenhut M, Riordan A. Mortality and morbidity of nosocomial respiratory syncytial virus (RSV) infection in ventilated children--a ten year perspective. Minerva Anestesiol 2012; 78(7): 782.

79. Pockett RD, Campbell D, Carroll S, Rajoriya F, Adlard N. Rotavirus, respiratory syncytial virus and non-rotaviral gastroenteritis analysis of hospital readmissions in England and Wales. Acta Paediatr 2013; 102(4): e158-e63.

80. Jacobs P, Lier D, Gooch K, Buesch K, Lorimer M, Mitchell I. A model of the costs of community and nosocomial pediatric respiratory syncytial virus infections in Canadian hospitals. Can J Infect Dis Med Microbiol 2013; 24(1): 22-6.

81. Mansbach JM, Emond JA, Camargo CA. Bronchiolitis in US emergency departments 1992 to 2000: epidemiology and practice variation. Pediatr Emerg Care 2005; 21(4): 242-7.

82. Mansbach JM, Pelletier AJ, Camargo CA. US outpatient office visits for bronchiolitis, 1993-2004. Ambul Pediatr 2007; 7(4): 304-7.

83. Hasegawa K, Tsugawa Y, Brown DF, Mansbach JM, Camargo CA. Temporal trends in emergency department visits for bronchiolitis in the United States, 2006 to 2010. Pediatr Infect Dis J 2014; 33(1): 11-8.

84. Rosychuk RJ, Klassen TP, Voaklander DC, Senthilselvan A, Rowe BH. Presentations of Infants to Emergency Departments in Alberta, Canada, for Bronchiolitis: A Large Population-Based Study. Pediatr Emerg Care 2011; 27(3).

85. Bourgeois FT, Valim C, McAdam AJ, Mandl KD. Relative Impact of Influenza and Respiratory Syncytial Virus in Young Children. Pediatrics 2009; 124(6): e1072-e80.

86. Johnson DW, Adair C, Brant R, Holmwood J, Mitchell I. Differences in Admission Rates of Children With Bronchiolitis by Pediatric and General Emergency Departments. Pediatrics 2002; 110(4): e49.

87. Garcia-Marcos L, Valverde-Molina J, Pavlovic-Nesic S, et al. Pediatricians' attitudes and costs of bronchiolitis in the emergency department: a prospective multicentre study. Pediatr Pulmonol 2014; 49(10): 1011-9.

88. Ehlken B, Ihorst G, Lippert B, et al. Economic impact of community-acquired and nosocomial lower respiratory tract infections in young children in Germany. Eur J Pediatr 2005; 164(10): 607-15.

89. Edwards KM, Thompson J, Paolini J, Wright PF. Adenovirus Infections in Young Children. Pediatrics 1985; 76(3): 420-4.

90. van den Hoogen BG, de Jong JC, Groen J, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med 2001; 7(6): 719-24.

91. Papadopoulos NG, Moustaki M, Tsolia M, et al. Association of Rhinovirus Infection with Increased Disease Severity in Acute Bronchiolitis. Am J Respir Crit Care Med 2002; 165(9): 1285-9.

92. Iwane MK, Edwards KM, Szilagyi PG, et al. Population-Based Surveillance for Hospitalizations Associated With Respiratory Syncytial Virus, Influenza Virus, and Parainfluenza Viruses Among Young Children. Pediatrics 2004; 113(6): 1758-64.

93. Jartti T, Lehtinen P, Vuorinen T, et al. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. Emerg Infect Dis 2004; 10(6): 1095-101.

94. Rocholl C, Gerber K, Daly J, Pavia AT, Byington CL. Adenoviral Infections in Children: The Impact of Rapid Diagnosis. Pediatrics 2004; 113(1): e51-e6.

95. Jartti T, Lehtinen P, Vuorinen T, Ruuskanen O. Bronchiolitis: age and previous wheezing episodes are linked to viral etiology and atopic characteristics. Pediatr Infect Dis J 2009; 28(4): 311-7.

96. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A 2005; 102(36): 12891-6.

97. Arnold JC, Singh KK, Spector SA, Sawyer MH. Human Bocavirus: Prevalence and Clinical Spectrum at a Children's Hospital. Clin Infect Dis 2006; 43(3): 283-8.

98. Ma X, Endo R, Ishiguro N, et al. Detection of Human Bocavirus in Japanese Children with Lower Respiratory Tract Infections. J Clin Microbiol 2006; 44(3): 1132-4.

99. van der Hoek L, Pyrc K, Jebbink MF, et al. Identification of a new human coronavirus. Nat Med 2004; 10(4): 368-73.

100. Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. Evidence of a Novel Human Coronavirus That Is Associated with Respiratory Tract Disease in Infants and Young Children. J Infect Dis 2005; 191(4): 492-8.

101. Woo PCY, Lau SKP, Chu C-m, et al. Characterization and Complete Genome Sequence of a Novel Coronavirus, Coronavirus HKU1, from Patients with Pneumonia. J Virol 2005; 79(2): 884-95.

102. Allander T, Andreasson K, Gupta S, et al. Identification of a Third Human Polyomavirus. J Virol 2007; 81(8): 4130-6.

103. Gaynor AM, Nissen MD, Whiley DM, et al. Identification of a Novel Polyomavirus from Patients with Acute Respiratory Tract Infections. PLoS Pathog 2007; 3(5): e64.

104. Kuypers J, Martin ET, Heugel J, Wright N, Morrow R, Englund JA. Clinical Disease in Children Associated With Newly Described Coronavirus Subtypes. Pediatrics 2007; 119(1): e70-e6.

105. Wattier RL, Vazquez M, Weibel C, et al. Role of human polyomaviruses in respiratory tract disease in young children. Emerg Infect Dis 2008; 14(11): 1766-8.

106. Hall CB, Powell KR, Schnabel KC, Gala CL, Pincus PH. Risk of secondary bacterial infection in infants hospitalized with respiratory syncytial viral infection. J Pediatr 1988; 113(2): 266-71.

107. Purcell K, Fergie J. Concurrent serious bacterial infections in 2396 infants and children hospitalized with respiratory syncytial virus lower respiratory tract infections. Arch Pediatr Adolesc Med 2002; 156(4): 322-4.

108. Duttweiler L, Nadal D, Frey B. Pulmonary and systemic bacterial co-infections in severe RSV bronchiolitis. Arch Dis Child 2004; 89(12): 1155-7.

109. Thorburn K, Harigopal S, Reddy V, Taylor N, van Saene HKF. High incidence of pulmonary bacterial co-infection in children with severe respiratory syncytial virus (RSV) bronchiolitis. Thorax 2006; 61(7): 611-5.

110. Ralston S, Hill V, Waters A. Occult serious bacterial infection in infants younger than 60 to 90 days with bronchiolitis: a systematic review. Arch Pediatr Adolesc Med 2011; 165(10): 951-6.

111. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory Syncytial Virus Infection in Elderly and High-Risk Adults. N Engl J Med 2005; 352(17): 1749-59.

112. Mahony JB. Detection of Respiratory Viruses by Molecular Methods. Clin Microbiol Rev 2008; 21(4): 716-47.

113. Miller EK, Edwards KM, Weinberg GA, et al. A novel group of rhinoviruses is associated with asthma hospitalizations. J Allergy Clin Immunol 2009; 123(1): 98-104 e1.

114. Miller EK. New Human Rhinovirus Species and Their Significance in Asthma Exacerbation and Airway Remodeling. Immunol Allergy Clin North Am 2010; 30(4): 541-52.

115. Miller EK, Bugna J, Libster R, et al. Human Rhinoviruses in Severe Respiratory Disease in Very Low Birth Weight Infants. Pediatrics 2012; 129(1): e60-e7.

116. Kieninger E, Fuchs O, Latzin P, Frey U, Regamey N. Rhinovirus infections in infancy and early childhood. Eur Respir J 2013; 41(2): 443-52.

117. Williams JV. Déjà Vu All Over Again: Koch's Postulates and Virology in the 21st Century. J Infect Dis 2010; 201(11): 1611-4.

118. Pavia AT. Viral Infections of the Lower Respiratory Tract: Old Viruses, New Viruses, and the Role of Diagnosis. Clin Infect Dis 2011; 52(suppl 4): S284-S9.

119. Kusel MMH, de Klerk NH, Holt PG, Kebadze T, Johnston SL, Sly PD. Role of Respiratory Viruses in Acute Upper and Lower Respiratory Tract Illness in the First Year of Life: A Birth Cohort Study. Pediatr Infect Dis J 2006; 25(8).

120. Martin ET, Fairchok MP, Kuypers J, et al. Frequent and Prolonged Shedding of Bocavirus in Young Children Attending Daycare. J Infect Dis 2010; 201(11): 1625-32.

121. Wurzel DF, Marchant JM, Clark JE, et al. Respiratory virus detection in nasopharyngeal aspirate versus bronchoalveolar lavage is dependent on virus type in children with chronic respiratory symptoms. J Clin Virol 2013; 58(4): 683-8.

122. Horvath AR, Lord SJ, StJohn A, et al. From biomarkers to medical tests: The changing landscape of test evaluation. Clin Chim Acta 2014; 427(0): 49-57.

123. Calvo C, Pozo F, García-García ML, et al. Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. Acta Pædiatrica 2010; 99(6): 883-7.

124. Mansbach JM, McAdam AJ, Clark S, et al. Prospective Multicenter Study of the Viral Etiology of Bronchiolitis in the Emergency Department. Acad Emerg Med 2008; 15(2): 111-8.

125. Pica N, Bouvier NM. Environmental factors affecting the transmission of respiratory viruses. Curr Opin Virol 2012; 2(1): 90-5.

126. Lessler J, Reich NG, Brookmeyer R, Perl TM, Nelson KE, Cummings DAT. Incubation periods of acute respiratory viral infections: a systematic review. Lancet Infect Dis 2009; 9(5): 291-300.

127. Antunes H, Rodrigues H, Silva N, et al. Etiology of bronchiolitis in a hospitalized pediatric population: prospective multicenter study. J Clin Virol 2010; 48(2): 134-6.

128. Flores P, Rebelo-de-Andrade H, Gonçalves P, et al. Bronchiolitis Caused by Respiratory Syncytial Virus in an Area of Portugal: Epidemiology, Clinical Features, and Risk Factors. Eur J Clin Microbiol Infect Dis 2004; 23(1): 39-45.

129. Antunes J, Chambel M, Borrego LM, Prates S, Loureiro V. Infecções respiratórias virais na criança. Acta Pediatr Port 2013; 44(1): 9-14.

130. Stensballe LG, Devasundaram JK, Simoes EAF. Respiratory syncytial virus epidemics: the ups and downs of a seasonal virus. Pediatr Infect Dis J 2003; 22(2): S21-S32.

131. Panozzo CA, Fowlkes AL, Anderson LJ. Variation in Timing of Respiratory Syncytial Virus Outbreaks: Lessons From National Surveillance. Pediatr Infect Dis J 2007; 26(11): S41-S5.

132. Meerhoff TJ, Mosnier A, Schellevis F, Paget WJ. Progress in the surveillance of respiratory syncytial virus (RSV) in Europe: 2001-2008. Euro Surveill 2009; 14(40).

133. Calvo C, García-García ML, Blanco C, Pozo F, Flecha IC, Pérez-Breña P. Role of Rhinovirus in Hospitalized Infants With Respiratory Tract Infections in Spain. Pediatr Infect Dis J 2007; 26(10).

134. Johnston SL, Pattemore PK, Sanderson G, et al. The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis. Am J Respir Crit Care Med 1996; 154(3): 654-60.

135. Knott AM, Long CE, Hall CB. Parainfluenza viral infections in pediatric outpatients: seasonal patterns and clinical characteristics. Pediatr Infect Dis J 1994; 13(4): 269-73.

136. Welliver R. The relationship of meteorological conditions to the epidemic activity of respiratory syncytial virus. Paediatr Respir Rev 2009; 10: 6-8.

137. White LJ, Mandl JN, Gomes MG, et al. Understanding the transmission dynamics of respiratory syncytial virus using multiple time series and nested models. Math Biosci 2007; 209(1): 222-39.

138. Moore HC, Jacoby P, Hogan AB, Blyth CC, Mercer GN. Modelling the Seasonal Epidemics of Respiratory Syncytial Virus in Young Children. PLoS ONE 2014; 9(6): e100422.

139. Garofalo RP, Hintz KH, Hill V, Patti J, Ogra PL, Welliver RC. A comparison of epidemiologic and immunologic features of bronchiolitis caused by influenza virus and respiratory syncytial virus. J Med Virol 2005; 75(2): 282-9.

140. Gill MA, Long K, Kwon T, et al. Differential Recruitment of Dendritic Cells and Monocytes to Respiratory Mucosal Sites in Children with Influenza Virus or Respiratory Syncytial Virus Infection. J Infect Dis 2008; 198(11): 1667-76.

141. Welliver TP, Reed JL, Welliver RC, Sr. Respiratory syncytial virus and influenza virus infections: observations from tissues of fatal infant cases. Pediatr Infect Dis J 2008; 27(10 Suppl): S92-6.

142. Bont L. Current concepts of the pathogenesis of RSV bronchiolitis. Adv Exp Med Biol 2009; 634: 31-40.

143. Openshaw PJM. A Gene Expression Signature for RSV: Clinical Implications and Limitations. PLoS Med 2013; 10(11): e1001550.

144. Thorburn K, McNamara P. Bronchiolitis. In: Wheeler DS, Wong HR, Shanley TP, eds. Pediatric Critical Care Medicine: Springer London; 2014: 75-85.

145. Johnson JE, Gonzales RA, Olson SJ, Wright PF, Graham BS. The histopathology of fatal untreated human respiratory syncytial virus infection. Mod Pathol 2007; 20(1): 108-19.

146. Welliver TP, Garofalo RP, Hosakote Y, et al. Severe Human Lower Respiratory Tract Illness Caused by Respiratory Syncytial Virus and Influenza Virus Is Characterized by the Absence of Pulmonary Cytotoxic Lymphocyte Responses. J Infect Dis 2007; 195(8): 1126-36.

147. Bem RA, Domachowske JB, Rosenberg HF. Animal models of human respiratory syncytial virus disease. Am J Physiol Lung Cell Mol Physiol 2011; 301(2): L148-56.

148. Hall CB, Douglas RG, Jr., Geiman JM. Respiratory syncytial virus infections in infants: quantitation and duration of shedding. J Pediatr 1976; 89(1): 11-5.

149. Richardson LS, Belshe RB, Sly DL, et al. Experimental respiratory syncytial virus pneumonia in cebus monkeys. J Med Virol 1978; 2(1): 45-59.

150. Eisenhut M. Extrapulmonary manifestations of severe respiratory syncytial virus infection--a systematic review. Crit Care 2006; 10(4): R107.

151. Cirino NM, Panuska JR, Villani A, et al. Restricted replication of respiratory syncytial virus in human alveolar macrophages. Journal of General Virology 1993; 74(8): 1527-37.

152. Jones A, Morton I, Hobson L, Evans GS, Everard ML. Differentiation and immune function of human dendritic cells following infection by respiratory syncytial virus. Clin Exp Immunol 2006; 143(3): 513-22.

153. Halfhide CP, Brearey SP, Flanagan BF, et al. Neutrophil TLR4 expression is reduced in the airways of infants with severe bronchiolitis. Thorax 2009; 64(9): 798-805.

154. Collins P, Fearns R, Graham B. Respiratory Syncytial Virus: Virology, Reverse Genetics, and Pathogenesis of Disease. In: Anderson LJ, Graham BS, eds. Challenges and Opportunities for Respiratory Syncytial Virus Vaccines: Springer Berlin Heidelberg; 2013: 3-38.

155. McLellan J, Ray W, Peeples M. Structure and Function of Respiratory Syncytial Virus Surface Glycoproteins. In: Anderson LJ, Graham BS, eds. Challenges and Opportunities for Respiratory Syncytial Virus Vaccines: Springer Berlin Heidelberg; 2013: 83-104.

156. Triantafilou K, Kar S, Vakakis E, Kotecha S, Triantafilou M. Human respiratory syncytial virus viroporin SH: a viral recognition pathway used by the host to signal inflammasome activation. Thorax 2013; 68(1): 66-75.

157. Cox DW, Le Souef PN. Rhinovirus and the developing lung. Paediatr Respir Rev 2014; 15(3): 268-74.

158. Papadopoulos NG, Bates PJ, Bardin PG, et al. Rhinoviruses Infect the Lower Airways. J Infect Dis 2000; 181(6): 1875-84.

159. Malmstrom K, Pitkaranta A, Carpen O, et al. Human rhinovirus in bronchial epithelium of infants with recurrent respiratory symptoms. J Allergy Clin Immunol 2006; 118(3): 591-6.

160. Melero J, Moore M. Influence of Respiratory Syncytial Virus Strain Differences on Pathogenesis and Immunity. In: Anderson LJ, Graham BS, eds. Challenges and Opportunities for Respiratory Syncytial Virus Vaccines: Springer Berlin Heidelberg; 2013: 59-82.

161. Buckingham SC, Bush AJ, Devincenzo JP. Nasal quantity of respiratory syncytical virus correlates with disease severity in hospitalized infants. Pediatr Infect Dis J 2000; 19(2): 113-7.

162. Bont L, Kimpen JL. Immunological mechanisms of severe respiratory syncytial virus bronchiolitis. Intensive Care Med 2002; 28(5): 616-21.

163. DeVincenzo JP, El Saleeby CM, Bush AJ. Respiratory Syncytial Virus Load Predicts Disease Severity in Previously Healthy Infants. J Infect Dis 2005; 191(11): 1861-8.

164. DeVincenzo JP, Wilkinson T, Vaishnaw A, et al. Viral Load Drives Disease in Humans Experimentally Infected with Respiratory Syncytial Virus. Am J Respir Crit Care Med 2010; 182(10): 1305-14.

165. Houben ML, Coenjaerts FEJ, Rossen JWA, et al. Disease severity and viral load are correlated in infants with primary respiratory syncytial virus infection in the community. J Med Virol 2010; 82(7): 1266-71.

166. El Saleeby CM, Bush AJ, Harrison LM, Aitken JA, DeVincenzo JP. Respiratory Syncytial Virus Load, Viral Dynamics, and Disease Severity in Previously Healthy Naturally Infected Children. J Infect Dis 2011; 204(7): 996-1002.

167. Everard ML, Milner AD. The respiratory syncitial virus and its role in acute bronchiolitis. Eur J Pediatr 1992; 151(9): 638-51.

168. Brearey SP, Smyth RL. Pathogenesis of RSV in Children. In: Patricia C, ed. Perspectives in Medical Virology: Elsevier; 2006: 141-62.

169. Openshaw PJ, Chiu C. Protective and dysregulated T cell immunity in RSV infection. Curr Opin Virol 2013; 3(4): 468-74.

170. Mukherjee S, Lukacs N. Innate Immune Responses to Respiratory Syncytial Virus Infection. In: Anderson LJ, Graham BS, eds. Challenges and Opportunities for Respiratory Syncytial Virus Vaccines: Springer Berlin Heidelberg; 2013: 139-54.

171. Schwarze J, Mackenzie KJ. Novel insights into immune and inflammatory responses to respiratory viruses. Thorax 2013; 68(1): 108-10.

172. McNamara PS, Flanagan BF, Selby AM, Hart CA, Smyth RL. Pro- and anti-inflammatory responses in respiratory syncytial virus bronchiolitis. Eur Respir J 2004; 23(1): 106-12.

173. McNamara PS, Flanagan BF, Hart CA, Smyth RL. Production of Chemokines in the Lungs of Infants with Severe Respiratory Syncytial Virus Bronchiolitis. J Infect Dis 2005; 191(8): 1225-32.

174. McNamara PS, Fonceca AM, Howarth D, et al. Respiratory syncytial virus infection of airway epithelial cells, in vivo and in vitro, supports pulmonary antibody responses by inducing expression of the B cell differentiation factor BAFF. Thorax 2013; 68(1): 76-81.

175. McNamara PS, Ritson P, Selby A, Hart CA, Smyth RL. Bronchoalveolar lavage cellularity in infants with severe respiratory syncytial virus bronchiolitis. Arch Dis Child 2003; 88(10): 922-6.

176. Bont L, Heijnen CJ, Kavelaars A, et al. Peripheral blood cytokine responses and disease severity in respiratory syncytial virus bronchiolitis. Eur Respir J 1999; 14(1): 144-9.

177. Sheeran P, Jafri H, Carubelli C, et al. Elevated cytokine concentrations in the nasopharyngeal and tracheal secretions of children with respiratory syncytial virus disease. Pediatr Infect Dis J 1999; 18(2): 115-22.

178. Bont L, Kavelaars A, Heijnen CJ, van Vught AJ, Kimpen JL. Monocyte interleukin-12 production is inversely related to duration of respiratory failure in respiratory syncytial virus bronchiolitis. J Infect Dis 2000; 181(5): 1772-5.

179. Bont L, Heijnen Cobi J, Kavelaars A, et al. Local Interferon−γ Levels during Respiratory Syncytial Virus Lower Respiratory Tract Infection Are Associated with Disease Severity. J Infect Dis 2001; 184(3): 355-8.

180. Garofalo RP, Patti J, Hintz KA, Hill V, Ogra PL, Welliver RC. Macrophage Inflammatory Protein-1α (Not T Helper Type 2 Cytokines) Is Associated with Severe Forms of Respiratory Syncytial Virus Bronchiolitis. J Infect Dis 2001; 184(4): 393-9.

181. Noah TL, Ivins SS, Murphy P, Kazachkova I, Moats-Staats B, Henderson FW. Chemokines and inflammation in the nasal passages of infants with respiratory syncytial virus bronchiolitis. Clin Immunol 2002; 104(1): 86-95.

182. Tripp RA, Moore D, Barskey A, et al. Peripheral Blood Mononuclear Cells from Infants Hospitalized Owing to Respiratory Syncytial Virus Infection Express T Helper-1 and T Helper-2 Cytokines and CC Chemokine Messenger RNA. J Infect Dis 2002; 185(10): 1388-94.

183. Laham FR, Israele V, Casellas JM, et al. Differential Production of Inflammatory Cytokines in Primary Infection with Human Metapneumovirus and with Other Common Respiratory Viruses of Infancy. J Infect Dis 2004; 189(11): 2047-56.

184. Alonso Fernandez J, Roine I, Vasquez A, Caneo M. Soluble interleukin-2 receptor (sCD25) and interleukin-10 plasma concentrations are associated

with severity of primary respiratory syncytial virus (RSV) infection. Eur Cytokine Netw 2005; 16(1): 81-90.

185. Pinto RA, Arredondo SM, Bono MR, Gaggero AA, Díaz PV. T Helper 1/T Helper 2 Cytokine Imbalance in Respiratory Syncytial Virus Infection Is Associated With Increased Endogenous Plasma Cortisol. Pediatrics 2006; 117(5): e878-e86.

186. Juntti H, Osterlund P, Kokkonen J, et al. Cytokine responses in cord blood predict the severity of later respiratory syncytial virus infection. J Allergy Clin Immunol 2009; 124(1): 52-8.e1-2.

187. Bendelja K, Vojvoda V, Aberle N, et al. Decreased Toll-like receptor 8 expression and lower TNF-alpha synthesis in infants with acute RSV infection. Respir Res 2010; 11: 143.

188. García C, Soriano-Fallas A, Lozano J, et al. Decreased Innate Immune Cytokine Responses Correlate With Disease Severity in Children With Respiratory Syncytial Virus and Human Rhinovirus Bronchiolitis. Pediatr Infect Dis J 2012; 31(1).

189. Mella C, Suarez-Arrabal MC, Lopez S, et al. Innate immune dysfunction is associated with enhanced disease severity in infants with severe respiratory syncytial virus bronchiolitis. J Infect Dis 2013; 207(4): 564-73.

190. Barik S. Respiratory Syncytial Virus Mechanisms to Interfere with Type 1 Interferons. In: Anderson LJ, Graham BS, eds. Challenges and Opportunities for Respiratory Syncytial Virus Vaccines: Springer Berlin Heidelberg; 2013: 173-91.

191. Guerrero-Plata A, Casola A, Garofalo RP. Human Metapneumovirus Induces a Profile of Lung Cytokines Distinct from That of Respiratory Syncytial Virus. J Virol 2005; 79(23): 14992-7.

192. Guerrero-Plata A, Casola A, Suarez G, et al. Differential Response of Dendritic Cells to Human Metapneumovirus and Respiratory Syncytial Virus. Am J Respir Cell Mol Biol 2006; 34(3): 320-9.

193. Melendi GA, Laham FR, Monsalvo AC, et al. Cytokine Profiles in the Respiratory Tract During Primary Infection With Human Metapneumovirus, Respiratory Syncytial Virus, or Influenza Virus in Infants. Pediatrics 2007; 120(2): e410-e5.

194. Hartmann F, Fiori HH, Ramos Garcia PC, Piva J, Fiori RM. Surfactant Deficiency in Infants with Severe Acute Viral Bronchiolitis. J Pediatr 2014; 164(6): 1432-5.

195. Dargaville PA, South M, McDougall PN. Surfactant abnormalities in infants with severe viral bronchiolitis. Arch Dis Child 1996; 75(2): 133-6.

196. LeVine AM, Lotze A, Stanley S, et al. Surfactant content in children with inflammatory lung disease. Crit Care Med 1996; 24(6).

197. Skelton R, Holland P, Darowski M, Chetcuti PA, Morgan LW, Harwood JL. Abnormal surfactant composition and activity in severe bronchiolitis. Acta Pædiatrica 1999; 88(9): 942-6.

198. Ogilvie MM, Vathenen AS, Radford M, Codd J, Key S. Maternal antibody and respiratory syncytial virus infection in infancy. J Med Virol 1981; 7(4): 263-71.

199. Ochola R, Sande C, Fegan G, et al. The Level and Duration of RSV-Specific Maternal IgG in Infants in Kilifi Kenya. PLoS ONE 2009; 4(12): e8088.

200. Andabaka T, Nickerson JW, Rojas-Reyes MX, Rueda JD, Bacic Vrca V, Barsic B. Monoclonal antibody for reducing the risk of respiratory syncytial virus infection in children. Cochrane Database Syst Rev 2013; 4: Cd006602.

201. Taylor G. Immunology of RSV. In: Patricia C, ed. Perspectives in Medical Virology: Elsevier; 2006: 43-88.

202. Malloy AW, Falsey A, Ruckwardt T. Consequences of Immature and Senescent Immune Responses for Infection with Respiratory Syncytial Virus. In: Anderson LJ, Graham BS, eds. Challenges and Opportunities for Respiratory Syncytial Virus Vaccines: Springer Berlin Heidelberg; 2013: 211-31.

203. Varga S, Braciale T. The Adaptive Immune Response to Respiratory Syncytial Virus. In: Anderson LJ, Graham BS, eds. Challenges and Opportunities for Respiratory Syncytial Virus Vaccines: Springer Berlin Heidelberg; 2013: 155-71.

204. Nokes DJ, Okiro EA, Ngama M, et al. Respiratory Syncytial Virus Infection and Disease in Infants and Young Children Observed from Birth in Kilifi District, Kenya. Clin Infect Dis 2008; 46(1): 50-7.

205. Ohuma EO, Okiro EA, Ochola R, et al. The Natural History of Respiratory Syncytial Virus in a Birth Cohort: The Influence of Age and Previous Infection on Reinfection and Disease. Am J Epidemiol 2012; 176(9): 794-802.

206. Sande CJ, Mutunga MN, Okiro EA, Medley GF, Cane PA, Nokes DJ. Kinetics of the Neutralizing Antibody Response to Respiratory Syncytial Virus Infections in a Birth Cohort. J Med Virol 2013; 85(11): 2020-5.

207. Sande CJ, Cane PA, Nokes DJ. The association between age and the development of respiratory syncytial virus neutralising antibody responses following natural infection in infants. Vaccine 2014; 32(37):4726-9

208. Polack FP, Teng MN, L.Collins P, et al. A Role for Immune Complexes in Enhanced Respiratory Syncytial Virus Disease. J Exp Med 2002; 196(6): 859-65.

209. Delgado MF, Coviello S, Monsalvo AC, et al. Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. Nat Med 2009; 15(1): 34-41.

210. Varga SM. Fixing a failed vaccine. Nat Med 2009; 15(1): 21-2.

211. Janssen R, Bont L, Siezen CLE, et al. Genetic Susceptibility to Respiratory Syncytial Virus Bronchiolitis Is Predominantly Associated with Innate Immune Genes. J Infect Dis 2007; 196(6): 826-34.

212. Miyairi I, DeVincenzo JP. Human genetic factors and respiratory syncytial virus disease severity. Clin Microbiol Rev 2008; 21(4): 686-703.

213. Choi E, Lee H, Chanock S. Human Genetics and Respiratory Syncytial Virus Disease: Current Findings and Future Approaches. In: Anderson LJ, Graham BS, eds. Challenges and Opportunities for Respiratory Syncytial Virus Vaccines: Springer Berlin Heidelberg; 2013: 121-37.

214. Mansbach JM, Camargo CA. Bronchiolitis: Lingering Questions About Its Definition and the Potential Role of Vitamin D. Pediatrics 2008; 122(1): 177-9.

215. Belderbos ME, Houben ML, Wilbrink B, et al. Cord Blood Vitamin D Deficiency Is Associated With Respiratory Syncytial Virus Bronchiolitis. Pediatrics 2011; 127(6): e1513-e20.

216. Mansbach JM, Piedra PA, Borregaard N, et al. Serum cathelicidin level is associated with viral etiology and severity of bronchiolitis. J Allergy Clin Immunol 2012; 130(4): 1007-8.e1.

217. Stoppelenburg AJ, von Hegedus JH, Huis in't Veld R, Bont L, Boes M. Defective control of vitamin D receptor-mediated epithelial STAT1 signalling predisposes to severe respiratory syncytial virus bronchiolitis. J Pathol 2014; 232(1): 57-64.

218. Hattersley AT, McCarthy MI. What makes a good genetic association study? Lancet 2005; 366(9493): 1315-23.

219. Auffray C, Adcock IM, Chung KF, Djukanovic R, Pison C, Sterk PJ. AN integrative systems biology approach to understanding pulmonary diseases. Chest 2010; 137(6): 1410-6.

220. Wagener AH, Yick CY, Brinkman P, van der Schee MP, Fens N, Sterk PJ. Toward Composite Molecular Signatures in the Phenotyping of Asthma. Ann Am Thorac Soc 2013; 10(Supplement): S197-S205.

221. Wheelock CE, Goss VM, Balgoma D, et al. Application of 'omics technologies to biomarker discovery in inflammatory lung diseases. Eur Respir J 2013; 42(3): 802-25.

222. Schuurhof A, Bont L, Pennings JLA, et al. Gene Expression Differences in Lungs of Mice during Secondary Immune Responses to Respiratory Syncytial Virus Infection. J Virol 2010; 84(18): 9584-94.

223. Pennings JLA, Schuurhof A, Hodemaekers HM, et al. Systemic Signature of the Lung Response to Respiratory Syncytial Virus Infection. PLoS ONE 2011; 6(6): e21461.

224. Atzei A, Atzori L, Moretti C, et al. Metabolomics in paediatric respiratory diseases and bronchiolitis. J Matern Fetal Neonatal Med 2011; 24 Suppl 2: 59-62.

225. Kaminsky DA, Irvin CG, Sterk PJ. Complex systems in pulmonary medicine: a systems biology approach to lung disease. J Appl Physiol 2011; 110(6): 1716-22.

226. Ioannidis JPA. Expectations, validity, and reality in omics. J Clin Epidemiol 2010; 63(9): 945-9.

227. Committee on the Review of Omics-Based Tests for Predicting Patient Outcomes in Clinical T, Board on Health Care S, Board on Health Sciences P, Institute of M. In: Micheel CM, Nass SJ, Omenn GS, eds. Evolution of Translational Omics: Lessons Learned and the Path Forward. Washington (DC): National Academies Press (US)

228. Phelan PD, Williams HE, Freeman M. The Disturbances Of Ventilation In Acute Viral Bronchiolitis. J Paediatr Child Health 1968; 4(2): 96-104.

229. Stokes GM, Milner AD, Groggins RC. Work Of Breathing, Intra-Thoracic Pressure And Clinical Findings In A Group Of Babies With Bronchiolitis. Acta Pædiatrica 1981; 70(5): 689-94.

230. Hammer J, Numa A, Newth CJL. Acute respiratory distress syndrome caused by respiratory syncytial virus. Pediatr Pulmonol 1997; 23(3): 176-83.

231. Hislop AA, Wigglesworth JS, Desai R. Alveolar development in the human fetus and infant. Early Hum Dev 1986; 13(1): 1-11.

232. Hislop AA, Haworth SG. Airway size and structure in the normal fetal and infant lung and the effect of premature delivery and artificial ventilation. Am Rev Respir Dis 1989; 140(6): 1717-26.

233. Stocks J, Hislop AA. Structure and function of the respiratory system. Drug delivery to the lung 2002; 47-104.

234. Gappa M, Stocks J, Frey U. Assessing lung growth and function in infants and young children. Eur Respir Mon 2006; 37: 22–40.

235. Gaultier C, Denjean A. Chapter 3 - Developmental Anatomy and Physiology of the Respiratory System. In: Taussig LM, Landau LI, eds. Pediatric Respiratory Medicine (Second Edition). Philadelphia: Mosby; 2008: 15-34.

236. Stocks J, Hislop A, Sonnappa S. Early lung development: lifelong effect on respiratory health and disease. Lancet Respir Med 2013; 1(9): 728-42.

237. West JB. Respiratory physiology : the essentials. Philadelphia: Lippincott Williams & Wilkins; 2005.

238. Hogg JC, Williams J, Richardson JB, Macklem PT, Thurlbeck WM. Age as a Factor in the Distribution of Lower-Airway Conductance and in the Pathologic Anatomy of Obstructive Lung Disease. N Engl J Med 1970; 282(23): 1283-7.

239. Panitch HB, Deoras KS, Wolfson MR, Shaffer TH. Maturational Changes in Airway Smooth Muscle Structure-Function Relationships. Pediatr Res 1992; 31(2): 151-6.

240. Frey U, Jackson AC, Silverman M. Differences in airway wall compliance as a possible mechanism for wheezing disorders in infants. Eur Respir J 1998; 12(1): 136-42.

241. Smith TC, Marini JJ. Impact of PEEP on lung mechanics and work of breathing in severe airflow obstruction. J Appl Physiol 1988; 65(4): 1488-99.

242. Cambonie G, Milési C, Jaber S, et al. Nasal continuous positive airway pressure decreases respiratory muscles overload in young infants with severe acute viral bronchiolitis. Intensive Care Med 2008; 34(10): 1865-72.

243. Essouri S, Durand P, Chevret L, et al. Optimal level of nasal continuous positive airway pressure in severe viral bronchiolitis. Intensive Care Med 2011; 37(12): 2002-7.

244. Wheeler D. Life-Threatening Diseases of the Upper Respiratory Tract. In: Wheeler DS, Wong HR, Shanley TP, eds. Pediatric Critical Care Medicine: Springer London; 2014: 19-39.

245. McFadyen JG, Thompson D, Martin L. Applied Respiratory Physiology. In: Wheeler DS, Wong HR, Shanley TP, eds. Pediatric Critical Care Medicine: Springer London; 2014: 3-18.

246. Javouhey E, Pouyau R, Massenavette B. Pathophysiology of Acute Respiratory Failure in Children with Bronchiolitis and Effect of CPAP. In: Esquinas AM, ed. Noninvasive Ventilation in High-Risk Infections and Mass Casualty Events: Springer Vienna; 2014: 233-49.

247. Agostoni E. Volume-pressure relationships of the thorax and lung in the newborn. J Appl Physiol 1959; 14(6): 909-13.

248. Helms P, Beardsmore CS, Stocks J. Absolute intraesophageal pressure at functional residual capacity in frequency. J Appl Physiol 1981; 51(2): 270-5.

249. Papastamelos C, Panitch HB, England SE, Allen JL. Developmental changes in chest wall compliance in infancy and early childhood. J Appl Physiol 1995; 78(1): 179-84.

250. Kosch PC, Stark AR. Dynamic maintenance of end-expiratory lung volume in full-term infants. J Appl Physiol 1984; 57(4): 1126-33.

251. Stark AR, Cohlan BA, Waggener TB, Frantz ID, Kosch PC. Regulation of end-expiratory lung volume during sleep in premature infants. J Appl Physiol 1987; 62(3): 1117-23.

252. Henschen M, Stocks J, Brookes I, Frey U. New aspects of airway mechanics in pre-term infants. Eur Respir J 2006; 27(5): 913-20.

253. Hülskamp G, Pillow JJ, Dinger J, Stocks J. Lung Function Tests in Neonates and Infants with Chronic Lung Disease of Infancy: Functional Residual Capacity. Pediatr Pulmonol 2006; 41(1): 1-22.

254. Colin AA, McEvoy C, Castile RG. Respiratory Morbidity and Lung Function in Preterm Infants of 32 to 36 Weeks' Gestational Age. Pediatrics 2010; 126(1): 115-28.

255. Keens TG, Bryan AC, Levison H, Ianuzzo CD. Developmental pattern of muscle fiber types in human ventilatory muscles. J Appl Physiol 1978; 44(6): 909-13.

256. Gaultier C. Respiratory muscle function in infants. Eur Respir J 1995; 8(1): 150-3.

257. Ralston S, Hill V. Incidence of Apnea in Infants Hospitalized with Respiratory Syncytial Virus Bronchiolitis: A Systematic Review. J Pediatr 2009; 155(5): 728-33.

258. Gaultier C, Gallego J. Development of respiratory control: Evolving concepts and perspectives. Respir Physiol Neurobiol 2005; 149(1–3): 3-15.

259. Stock C, Teyssier G, Pichot V, Goffaux P, Barthelemy J-C, Patural H. Autonomic dysfunction with early respiratory syncytial virus-related infection. Auton Neurosci 2010; 156(1–2): 90-5.

260. Roe M, O'Donnell DR, Tasker RC. Respiratory viruses in the intensive care unit. Paediatr Respir Rev 2003; 4(3): 166-71.

261. Kneyber MCJ, Plötz FB. Respiratory Syncytial Virus (RSV) in the Pediatric Intensive Care Unit. In: Vincent J-L, ed. Intensive Care Med: Springer New York; 2007: 145-56.

262. Luo G, Nkoy FL, Gesteland PH, Glasgow TS, Stone BL. A systematic review of predictive modeling for bronchiolitis. Int J Med Inform 2014; 83(10): 691-714.

263. Feinstein AR. Clinical epidemiology : the architecture of clinical research. Philadelphia: W.B. Saunders Co.; 1985.

264. Riley RD, Sauerbrei W, Altman DG. Prognostic markers in cancer: the evolution of evidence from single studies to meta-analysis, and beyond. Br J Cancer 2009; 100(8): 1219-29.

265. Bouwmeester W, Zuithoff NP, Mallett S, et al. Reporting and methods in clinical prediction research: a systematic review. PLoS Med 2012; 9(5): 1-12.

266. Hernán MA, Robins JM. Estimating causal effects from epidemiological data. J Epidemiol Community Health 2006; 60(7): 578-86.

267. Bloemers BLP, van Furth AM, Weijerman ME, et al. Down Syndrome: A Novel Risk Factor for Respiratory Syncytial Virus Bronchiolitis— A Prospective Birth-Cohort Study. Pediatrics 2007; 120(4): e1076-e81.

268. Bloemers BLP, van Bleek GM, Kimpen JLL, Bont L. Distinct Abnormalities in the Innate Immune System of Children with Down Syndrome. J Pediatr 2010; 156(5): 804-9.e5.

269. Hemingway H, Croft P, Perel P, et al. Prognosis research strategy (PROGRESS) 1: a framework for researching clinical outcomes. BMJ 2013; 346: e5595.

270. Steyerberg EW, Moons KGM, van der Windt DA, et al. Prognosis Research Strategy (PROGRESS) 3: Prognostic Model Research. PLoS Med 2013; 10(2): e1001381.

271. Rietveld E, Vergouwe Y, Steyerberg EW, et al. Hospitalization for Respiratory Syncytial Virus Infection in Young Children: Development of a Clinical Prediction Rule. Pediatr Infect Dis J 2006; 25(3):201-7

272. Sampalis JS, Langley J, Carbonell-Estrany X, et al. Development and validation of a risk scoring tool to predict respiratory syncytial virus hospitalization in premature infants born at 33 through 35 completed weeks of gestation. Med Decis Making 2008; 28(4): 471-80.

273. Simoes EA, Carbonell-Estrany X, Fullarton JR, et al. A predictive model for respiratory syncytial virus (RSV) hospitalisation of premature infants born at 33-35 weeks of gestational age, based on data from the Spanish FLIP Study. Respir Res 2008; 9: 78.

274. Paes B, Cole M, Latchman A, Pinelli J. Predictive value of the respiratory syncytial virus risk-scoring tool in the term infant in Canada. Curr Med Res Opin 2009; 25(9): 2191-6.

275. Carbonell-Estrany X, Simoes EA, Fullarton JR, Ferdynus C, Gouyon JB. Validation of a model to predict hospitalization due to RSV of infants born at 33-35 weeks' gestation. J Perinat Med 2010; 38(4): 411-7.

276. Stensballe LG, Fullarton JR, Carbonell-Estrany X, Simoes EA. Population based external validation of a European predictive model for respiratory syncytial virus hospitalization of premature infants born 33 to 35 weeks of gestational age. Pediatr Infect Dis J 2010; 29(4): 374-6.

277. Simoes EA, Carbonell-Estrany X, Fullarton JR, Rossi GA, Barberi I, Lanari M. European risk factors' model to predict hospitalization of premature infants born 33-35 weeks' gestational age with respiratory syncytial virus: validation with Italian data. J Matern Fetal Neonatal Med 2011; 24(1): 152-7.

278. Simoes EAF. Environmental and demographic risk factors for respiratory syncytial virus lower respiratory tract disease. J Pediatr 2003; 143(5): 118-26.

279. Carroll KN, Gebretsadik T, Griffin MR, et al. Maternal Asthma and Maternal Smoking Are Associated With Increased Risk of Bronchiolitis During Infancy. Pediatrics 2007; 119(6): 1104-12.

280. Stensballe LG, Hjuler T, Andersen A, et al. Hospitalization for Respiratory Syncytial Virus Infection and Invasive Pneumococcal Disease in Danish Children Aged <2 Years: A Population-Based Cohort Study. Clin Infect Dis 2008; 46(8): 1165-71.

281. Simões EAF, Mutyara K, Soh S, Agustian D, Hibberd ML, Kartasasmita CB. The Epidemiology of Respiratory Syncytial Virus Lower Respiratory Tract Infections in Children Less than 5 Years of Age in Indonesia. Pediatr Infect Dis J 2011; 30(9):778-84

282. Figueras-Aloy J, Carbonell-Estrany X, Quero J, Group IS. Case-Control Study of the Risk Factors Linked to Respiratory Syncytial Virus Infection Requiring Hospitalization in Premature Infants Born at a Gestational Age of 33–35 Weeks in Spain. Pediatr Infect Dis J 2004; 23(9):815-20.

283. Figueras-Aloy J, Carbonell-Estrany X, Quero-Jiménez J, et al. FLIP-2 Study: Risk Factors Linked to Respiratory Syncytial Virus Infection Requiring Hospitalization in Premature Infants Born in Spain at a Gestational Age of 32 to 35 Weeks. Pediatr Infect Dis J 2008; 27(9):788-93.

284. Bont L, Houben ML. Commentary: Why Are Young Healthy Term Infants Protected Against Respiratory Syncytial Virus Bronchiolitis? Pediatr Infect Dis J 2011; 30(9):785-6.

285. Martinez FD, Morgan WJ, Wright AL, Holberg CJ, Taussig LM. Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. N Engl J Med 1988; 319(17): 1112-7.

286. Houben ML, Rovers MM, Wilbrink B, et al. High Concentrations of Amniotic Fluid Proinflammatory Cytokines in Healthy Neonates Are Associated With Low Risk of Respiratory Syncytial Virus Bronchiolitis. Pediatr Infect Dis J 2012; 31(9):931-4.

287. Colosia AD, Masaquel A, Hall CB, Barrett AM, Mahadevia PJ, Yogev R. Residential crowding and severe respiratory syncytial virus disease among infants and young children: a systematic literature review. BMC Infect Dis 2012; 12: 95.

288. Law BJ, Langley JM, Allen U, et al. The Pediatric Investigators Collaborative Network on Infections in Canada Study of Predictors of Hospitalization for Respiratory Syncytial Virus Infection for Infants Born at 33 Through 35 Completed Weeks of Gestation. Pediatr Infect Dis J 2004; 23(9):806-14.

289. Gouyon JB, RozÉ JC, Guillermet-Fromentin C, et al. Hospitalizations for respiratory syncytial virus bronchiolitis in preterm infants at <33 weeks gestation without bronchopulmonary dysplasia: the CASTOR study. Epidemiol Infect 2013; 141(04): 816-26.

290. DiFranza JR, Masaquel A, Barrett AM, Colosia AD, Mahadevia PJ. Systematic literature review assessing tobacco smoke exposure as a risk factor for serious respiratory syncytial virus disease among infants and young children. BMC Pediatr 2012; 12: 81.

291. Semple MG, Taylor-Robinson DC, Lane S, Smyth RL. Household Tobacco Smoke and Admission Weight Predict Severe Bronchiolitis in Infants Independent of Deprivation: Prospective Cohort Study. PLoS ONE 2011; 6(7): e22425.

292. Le Souëf PN. Tobacco related lung diseases begin in childhood. Thorax 2000; 55(12): 1063-7.

293. Hofhuis W, de Jongste JC, Merkus PJFM. Adverse health effects of prenatal and postnatal tobacco smoke exposure on children. Arch Dis Child 2003; 88(12): 1086-90.

294. Stocks J, Dezateux C. The effect of parental smoking on lung function and development during infancy. Respirology 2003; 8(3): 266-85.

295. Committee on Environmental Health, Committee on Substance Abuse Committee on Adolescence and Committee on Native American Child Health. Tobacco Use: A Pediatric Disease. Pediatrics 2009; 124(5): 1474-87.

296. Jones LL, Hashim A, McKeever T, Cook DG, Britton J, Leonardi-Bee J. Parental and household smoking and the increased risk of bronchitis, bronchiolitis and other lower respiratory infections in infancy: systematic review and meta-analysis. Respir Res 2011; 12: 5.

297. Bachrach V, Schwarz E, Bachrach L. Breastfeeding and the risk of hospitalization for respiratory disease in infancy: A meta-analysis. Arch Pediatr Adolesc Med 2003; 157(3): 237-43.

298. Ip S, Chung M, Raman G, et al. Breastfeeding and maternal and infant health outcomes in developed countries. Evidence report/technology assessment 2007; (153): 1-186.

299. Duijts L, Ramadhani MK, Moll HA. Breastfeeding protects against infectious diseases during infancy in industrialized countries. A systematic review. Matern Child Nutr 2009; 5(3): 199-210.

300. Nishimura T, Suzue J, Kaji H. Breastfeeding reduces the severity of respiratory syncytial virus infection among young infants: A multi-center prospective study. Pediatr Int 2009; 51(6): 812-6.

301. Lanari M, Prinelli F, Adorni F, et al. Maternal milk protects infants against bronchiolitis during the first year of life. Results from an Italian cohort of newborns. Early Hum Dev 2013; 89, Supplement 1(0): S51-S7.

302. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. Cochrane Database Syst Rev 2012; 8: Cd003517.

303. Brew BK, Allen CW, Toelle BG, Marks GB. Systematic review and meta-analysis investigating breast feeding and childhood wheezing illness. Paediatr Perinat Epidemiol 2011; 25(6): 507-18.

304. Matheson MC, Allen KJ, Tang MLK. Understanding the evidence for and against the role of breastfeeding in allergy prevention. Clin Exp Allergy 2012; 42(6): 827-51.

305. Dogaru CM, Nyffenegger D, Pescatore AM, Spycher BD, Kuehni CE. Breastfeeding and Childhood Asthma: Systematic Review and Meta-Analysis. Am J Epidemiol 2014; 179(10): 1153-67.

306. Kramer MS. Invited Commentary: Does Breastfeeding Protect Against "Asthma"? Am J Epidemiol 2014; 179(10): 1168-70.

307. Belderbos ME, Houben ML, van Bleek GM, et al. Breastfeeding modulates neonatal innate immune responses: a prospective birth cohort study. Pediatr Allergy Immunol 2012; 23(1): 65-74.

308. Dagan R, Landau D, Haikin H, Tal A. Hospitalization of Jewish and Bedouin infants in southern Israel for bronchiolitis caused by respiratory syncytial virus. Pediatr Infect Dis J 1993; 12(5): 381-6.

309. Hayes EB, Hurwitz ES, Schonberger LB, Anderson LJ. Respiratory syncytial virus outbreak on American Samoa. Evaluation of risk factors. Am J Dis Child 1989; 143(3): 316-21.

310. Karron RA, Singleton RJ, Bulkow L, et al. Severe Respiratory Syncytial Virus Disease in Alaska Native Children. J Infect Dis 1999; 180(1): 41-9.

311. Lowther SA, Shay DK, Holman RC, Clarke MJ, Kaufman SF, Anderson LJ. Bronchiolitis-associated hospitalizations among American Indian and Alaska Native children. Pediatr Infect Dis J 2000; 19(1).

312. Banerji A. High rates of hospitalisation for bronchiolitis in Inuit children on Baffin Island. Int J Circumpolar Health 2001; 60(3): 375-9.

313. Kovesi T. Respiratory disease in Canadian First Nations and Inuit children. Paediatr Child Health 2012; 17(7): 376-80.

314. Thorburn K, Kerr S, Taylor N, van Saene HK. RSV outbreak in a paediatric intensive care unit. J Hosp Infect 2004; 57(3): 194-201.

315. Medrano López C, García-Guereta L, Group CS. Community-acquired Respiratory Infections in Young Children With Congenital Heart Diseases in the Palivizumab Era: The Spanish 4-Season Civic Epidemiologic Study. Pediatr Infect Dis J 2010; 29(12).

316. Jobe AH, Bancalari E. Bronchopulmonary Dysplasia. Am J Respir Crit Care Med 2001; 163(7): 1723-9.

317. Manzoni P, Paes B, Resch B, Carbonell-Estrany X, Bont L. High risk for RSV bronchiolitis in late preterms and selected infants affected by rare disorders: a dilemma of specific prevention. Early Hum Dev 2012; 88 Suppl 2: S34-41.

318. Blanken MO, Rovers MM, Molenaar JM, et al. Respiratory syncytial virus and recurrent wheeze in healthy preterm infants. N Engl J Med 2013; 368(19): 1791-9.

319. Tissing WJE, van Steensel-Moll HA, Offringa M. Risk factors for mechanical ventilation in respiratory syncytial virus infection. Eur J Pediatr 1993; 152(2): 125-7.

320. Stocks J, Sonnappa S. Early life influences on the development of chronic obstructive pulmonary disease. Ther Adv Respir Dis 2013; 7(3): 161-73.

321. Eisenhut M, Sidaras D, Johnson R, Newland P, Thorburn K. Cardiac troponin T levels and myocardial involvement in children with severe respiratory syncytial virus lung disease. Acta Paediatr 2004; 93(7): 887-90.

322. Esposito S, Salice P, Bosis S, et al. Altered cardiac rhythm in infants with bronchiolitis and respiratory syncytial virus infection. BMC Infect Dis 2010; 10: 305.

323. Thorburn K, Eisenhut M, Shauq A, Narayanswamy S, Burgess M. Right ventricular function in children with severe respiratory syncytial virus (RSV) bronchiolitis. Minerva Anestesiol 2011; 77(1): 46-53.

324. Kristensen K, Hjuler T, Ravn H, Simões EAF, Stensballe LG. Chronic Diseases, Chromosomal Abnormalities, and Congenital Malformations as Risk Factors for Respiratory Syncytial Virus Hospitalization: A Population-Based Cohort Study. Clin Infect Dis 2012; 54(6): 810-7.

325. Wilkesmann A, Ammann RA, Schildgen O, et al. Hospitalized children with respiratory syncytial virus infection and neuromuscular impairment face an increased risk of a complicated course. Pediatr Infect Dis J 2007; 26(6): 485-91.

326. Simon A, Prusseit J, Muller A. Respiratory syncytial virus infection in children with neuromuscular impairment. Open Microbiol J 2011; 5: 155-8.

327. Simon A, Schildgen O, Schuster F. Viral infections in paediatric patients receiving conventional cancer chemotherapy. Arch Dis Child 2008; 93(10): 880-9.

328. Resch B, Manzoni P, Lanari M. Severe respiratory syncytial virus (RSV) infection in infants with neuromuscular diseases and immune deficiency syndromes. Paediatr Respir Rev 2009; 10(3): 148-53.

329. Asner S, Stephens D, Pedulla P, Richardson SE, Robinson J, Allen U. Risk factors and outcomes for respiratory syncytial virus-related infections in immunocompromised children. Pediatr Infect Dis J 2013; 32(10): 1073-6.

330. Lanari M, Vandini S, Capretti MG, Lazzarotto T, Faldella G. Respiratory Syncytial Virus Infections in Infants Affected by Primary Immunodeficiency. Journal of Immunology Research 2014; 2014: 6.

331. El Saleeby CM, Somes GW, DeVincenzo JP, Gaur AH. Risk Factors for Severe Respiratory Syncytial Virus Disease in Children With Cancer: The Importance of Lymphopenia and Young Age. Pediatrics 2008; 121(2): 235-43.

332. Zachariah P, Ruttenber M, Simões EAF. Down Syndrome and Hospitalizations due to Respiratory Syncytial Virus: A Population-Based Study. J Pediatr 2012; 160(5): 827-31.e1.

333. Fitzgerald P, Leonard H, Pikora TJ, Bourke J, Hammond G. Hospital Admissions in Children with Down Syndrome: Experience of a Population-Based Cohort Followed from Birth. PLoS ONE 2013; 8(8): e70401.

334. Bloemers BLP, Broers CJM, Bont L, Weijerman ME, Gemke RJBJ, van Furth AM. Increased risk of respiratory tract infections in children with Down syndrome: the consequence of an altered immune system. Microbes and Infection 2010; 12(11): 799-808.

335. Ram G, Chinen J. Infections and immunodeficiency in Down syndrome. Clin Exp Immunol 2011; 164(1): 9-16.

336. Simon A, Müller A, Khurana K, et al. Nosocomial infection: A risk factor for a complicated course in children with respiratory syncytial virus infection – Results from a prospective multicenter German surveillance study. Int J Hyg Environ Health 2008; 211(3–4): 241-50.

337. von Renesse A, Schildgen O, Klinkenberg D, Müller A, von Moers A, Simon A. Respiratory syncytial virus infection in children admitted to hospital but ventilated mechanically for other reasons. J Med Virol 2009; 81(1): 160-6.

338. Semple MG, Cowell A, Dove W, et al. Dual Infection of Infants by Human Metapneumovirus and Human Respiratory Syncytial Virus Is Strongly Associated with Severe Bronchiolitis. J Infect Dis 2005; 191(3): 382-6.

339. Caracciolo S, Minini C, Colombrita D, et al. Human metapneumovirus infection in young children hospitalized with acute respiratory tract disease: virologic and clinical features. Pediatr Infect Dis J 2008; 27(5): 406-12.

340. Midulla F, Scagnolari C, Bonci E, et al. Respiratory syncytial virus, human bocavirus and rhinovirus bronchiolitis in infants. Arch Dis Child 2010; 95(1): 35-41.

341. Marguet C, Lubrano M, Gueudin M, et al. In Very Young Infants Severity of Acute Bronchiolitis Depends On Carried Viruses. PLoS ONE 2009; 4(2): e4596.

342. Jartti T, Aakula M, Mansbach JM, et al. Hospital Length-of-stay Is Associated With Rhinovirus Etiology of Bronchiolitis. Pediatr Infect Dis J 2014; 33(8).

343. Greensill J, McNamara PS, Dove W, Flanagan B, Smyth RL, Hart CA. Human metapneumovirus in severe respiratory syncytial virus bronchiolitis. Emerg Infect Dis 2003; 9(3): 372-5.

344. Fletcher JN, Smyth RL, Thomas HM, Ashby D, Hart CA. Respiratory syncytial virus genotypes and disease severity among children in hospital. Arch Dis Child 1997; 77(6): 508-11.

345. Walsh EE, McConnochie KM, Long CE, Hall CB. Severity of respiratory syncytial virus infection is related to virus strain. J Infect Dis 1997; 175(4): 814-20.

346. Papadopoulos NG, Gourgiotis D, Javadyan A, et al. Does respiratory syncytial virus subtype influences the severity of acute bronchiolitis in hospitalized infants? Respir Med 2004; 98(9): 879-82.

347. Fodha I, Vabret A, Ghedira L, et al. Respiratory syncytial virus infections in hospitalized infants: association between viral load, virus subgroup, and disease severity. J Med Virol 2007; 79(12): 1951-8.

348. Kuehni CE, Spycher BD, Silverman M. Causal links between RSV infection and asthma: no clear answers to an old question. Am J Respir Crit Care Med. United States; 2009: 1079-80.

349. Bont L, Ramilo O. The relationship between RSV bronchiolitis and recurrent wheeze: the chicken and the egg. Early Hum Dev 2011; 87 Suppl 1: S51-4.

350. Cherry JD, Harrison GJ, Kaplan SL, Hotez PJ, Steinbach WJ. Feigin and Cherry's textbook of pediatric infectious diseases. 7th ed. Philadelphia: Saunders Elsevier; 2013.

351. Damore D, Mansbach JM, Clark S, Ramundo M, Camargo Jr CA. Prospective Multicenter Bronchiolitis Study: Predicting Intensive Care Unit Admissions. Acad Emerg Med 2008; 15(10): 887-94.

352. Corneli HM, Zorc JJ, Holubkov R, et al. Bronchiolitis: clinical characteristics associated with hospitalization and length of stay. Pediatr Emerg Care 2012; 28(2): 99-103.

353. Reilly CM, Stokes J, Jr., Mc CL, et al. Studies of acute respiratory illnesses caused by respiratory syncytial virus. 3. Clinical and laboratory findings. N Engl J Med 1961; 264: 1176-82.

354. Ebell MH. Risk stratification of children with bronchiolitis. Am Fam Physician 2012; 86(3): 275, 9.

355. Ochoa Sangrador C, Gonzalez de Dios J. [Consensus conference on acute bronchiolitis (VI): prognosis of acute bronchiolitis. Review of scientific evidence]. An Pediatr (Barc) 2010; 72(5): 354.e1-34.

356. Walsh P, Rothenberg SJ, O'Doherty S, Hoey H, Healy R. A validated clinical model to predict the need for admission and length of stay in children with acute bronchiolitis. Eur J Emerg Med 2004; 11(5): 265-72.

357. Shaw KN, Bell LM, Sherman NH. Outpatient assessment of infants with bronchiolitis. Am J Dis Child 1991; 145(2): 151-5.

358. Brooks A-M, McBride JT, McConnochie KM, Aviram M, Long C, Hall CB. Predicting Deterioration in Previously Healthy Infants Hospitalized With Respiratory Syncytial Virus Infection. Pediatrics 1999; 104(3): 463-7.

359. Voets S, van Berlaer G, Hachimi-Idrissi S. Clinical predictors of the severity of bronchiolitis. Eur J Emerg Med 2006; 13(3): 134-8.

360. Plint AC, Johnson DW, Correll R, et al. Predicting the Development of Severe Bronchiolitis Among an Emergency Department Cohort: A Multi-Center Study. Pediatric Academic Societies; 2008.

361. Marlais M, Evans J, Abrahamson E. Clinical predictors of admission in infants with acute bronchiolitis. Arch Dis Child 2011; 96(7): 648-52.

362. Weisgerber MC, Lye PS, Li S-H, et al. Factors predicting prolonged hospital stay for infants with bronchiolitis. J Hosp Med 2011; 6(5): 264-70.

363. McMillan JA, Tristram DA, Weiner LB, Higgins AP, Sandstrom C, Brandon R. Prediction of the duration of hospitalization in patients with respiratory syncytial virus infection: use of clinical parameters. Pediatrics 1988; 81(1): 22-6.

364. Mulholland EK, Olinsky A, Shann FA. Clinical findings and severity of acute bronchiolitis. Lancet 1990; 335(8700): 1259-61.

365. Lovett PB, Buchwald JM, Stürmann K, Bijur P. The vexatious vital: Neither clinical measurements by nurses nor an electronic monitor provides accurate measurements of respiratory rate in triage. Ann Emerg Med; 45(1): 68-76.

366. Simoes EA, Roark R, Berman S, Esler LL, Murphy J. Respiratory rate: measurement of variability over time and accuracy at different counting periods. Arch Dis Child 1991; 66(10): 1199-203.

367. Campbell H, Byass P, O'Dempsey TJ. Effects of body temperature on respiratory rate in young children. Arch Dis Child 1992; 67(5): 664.

368. O'Dempsey TJ, Laurence BE, McArdle TF, Todd JE, Lamont AC, Greenwood BM. The effect of temperature reduction on respiratory rate in febrile illnesses. Arch Dis Child 1993; 68(4): 492-5.

369. Rusconi F, Castagneto M, Porta N, et al. Reference Values for Respiratory Rate in the First 3 Years of Life. Pediatrics 1994; 94(3): 350-5.

370. Taylor JA, Beccaro M, Done S, Winters W. EStablishing clinically relevant standards for tachypnea in febrile children younger than 2 years. Arch Pediatr Adolesc Med 1995; 149(3): 283-7.

371. Davies P, Maconochie I. The relationship between body temperature, heart rate and respiratory rate in children. Emerg Med J 2009; 26(9): 641-3.

372. Nijman RG, Thompson M, van Veen M, Perera R, Moll HA, Oostenbrink R. Derivation and validation of age and temperature specific reference values and centile charts to predict lower respiratory tract infection in children with fever: prospective observational study. BMJ 2012; 345: e4224.

373. Lanaspa M, Valim C, Acacio S, et al. High Reliability in Respiratory Rate Assessment in Children with Respiratory Symptomatology in a Rural Area in Mozambique. J Trop Pediatr 2014; 60(2): 93-8.

374. Fleming S, Thompson M, Stevens R, et al. Normal ranges of heart rate and respiratory rate in children from birth to 18 years of age: a systematic review of observational studies. Lancet 2011; 377(9770): 1011-8.

375. Thompson M, Harnden A, Perera R, et al. Deriving temperature and age appropriate heart rate centiles for children with acute infections. Arch Dis Child 2009; 94(5): 361-5.

376. Parker MJ, Allen U, Stephens D, Lalani A, Schuh S. Predictors of major intervention in infants with bronchiolitis. Pediatr Pulmonol 2009; 44(4): 358-63.

377. Pasterkamp H, Kraman SS, Wodicka GR. Respiratory Sounds. Am J Respir Crit Care Med 1997; 156(3): 974-87.

378. Murphy RLH. In Defense of the Stethoscope. Respir Care 2008; 53(3): 355-69.

379. Mellis C. Respiratory noises: how useful are they clinically? Pediatr Clin North Am 2009; 56(1): 1-17, ix.

380. Sly PD, Collins RA. Physiological basis of respiratory signs and symptoms. Paediatr Respir Rev 2006; 7(2): 84-8.

381. Elphick HE, Lancaster GA, Solis A, Majumdar A, Gupta R, Smyth RL. Validity and reliability of acoustic analysis of respiratory sounds in infants. Arch Dis Child 2004; 89(11): 1059-63.

382. Schroeder AR, Mansbach JM, Stevenson M, et al. Apnea in Children Hospitalized With Bronchiolitis. Pediatrics 2013; 132(5): e1194-e201.

383. Rayyan M, Naulaers G, Daniels H, Allegaert K, Debeer A, Devlieger H. Characteristics of respiratory syncytial virus-related apnoea in three infants. Acta Paediatr 2004; 93(6): 847-9.

384. Ricart S, Rovira N, Garcia-Garcia JJ, et al. Frequency of Apnea and Respiratory Viruses in Infants with Bronchiolitis. Pediatr Infect Dis J 2014; 33(9):988-90.

385. El-Radhi AS, Barry W, Patel S. Association of fever and severe clinical course in bronchiolitis. Arch Dis Child 1999; 81(3): 231-4.

386. Ricart S, Marcos MA, Sarda M, et al. Clinical risk factors are more relevant than respiratory viruses in predicting bronchiolitis severity. Pediatr Pulmonol 2013; 48(5): 456-63.

387. Maneker AJ, Petrack EM, Krug SE. Contribution of Routine Pulse Oximetry to Evaluation and Management of Patients With Respiratory Illness in a Pediatric Emergency Department. Ann Emerg Med 1995; 25(1): 36-40.

388. Mower WR, Sachs C, Nicklin EL, Baraff LJ. Pulse Oximetry as a Fifth Pediatric Vital Sign. Pediatrics 1997; 99(5): 681-6.

389. Wang EE, Law BJ, Stephens D. Pediatric Investigators Collaborative Network on Infections in Canada (PICNIC) prospective study of risk factors and outcomes in patients hospitalized with respiratory syncytial viral lower respiratory tract infection. J Pediatr 1995; 126(2): 212-9.

390. Schroeder AR, Marmor AK, Pantell RH, Newman TB. Impact of pulse oximetry and oxygen therapy on length of stay in bronchiolitis hospitalizations. Arch Pediatr Adolesc Med 2004; 158(6): 527-30.

391. Brown L, Reiley DG, Jeng A, Green SM. Bronchiolitis: Can objective criteria predict eligibility for brief hospitalization? CJEM 2003; 5(4): 239-44.

392. Unger S, Cunningham S. Effect of Oxygen Supplementation on Length of Stay for Infants Hospitalized With Acute Viral Bronchiolitis. Pediatrics 2008; 121(3): 470-5.

393. Scottish Intercollegiate Guidelines Network. Bronchiolitis in children. A national clinical guideline. 2006. http://www.sign.ac.uk/pdf/sign91.pdf.

394. Subcommittee on Diagnosis and Management of B. Diagnosis and Management of Bronchiolitis. Pediatrics 2006; 118(4): 1774-93.

395. Mallory MD, Shay DK, Garrett J, Bordley WC. Bronchiolitis Management Preferences and the Influence of Pulse Oximetry and Respiratory Rate on the Decision to Admit. Pediatrics 2003; 111(1): e45-e51.

396. Cunningham S, McMurray A. Observational study of two oxygen saturation targets for discharge in bronchiolitis. Arch Dis Child 2012; 97(4): 361-3.

397. Schuh S, Freedman S, Coates A, et al. Effect of oximetry on hospitalization in bronchiolitis: a randomized clinical trial. J Am Med Assoc 2014; 312(7): 712-8.

398. Monitoring OXYgen in Infants Hospitalized With Bronchiolitis: A Best Practices Trial (The MOXY Trial). NCT01646606. https://clinicaltrials.gov/ct2/show/NCT01646606

399. Bronchiolitis of Infancy Discharge Study: a multi-centre, parallel-group, double blind, randomised controlled, equivalence study. ISRCTN28405428. http://www.isrctn.com/ISRCTN28405428

400. Bordley WC, Viswanathan M, King VJ, et al. Diagnosis and testing in bronchiolitis: a systematic review. Arch Pediatr Adolesc Med 2004; 158(2): 119-26.

401. Vogel AM, Lennon DR, Harding JE, et al. Variations in bronchiolitis management between five New Zealand hospitals: can we do better? J Paediatr Child Health 2003; 39(1): 40-5.

402. Johnson LW, Robles J, Hudgins A, Osburn S, Martin D, Thompson A. Management of bronchiolitis in the emergency department: impact of evidence-based guidelines? Pediatrics 2013; 131 Suppl 1: S103-9.

403. Knapp JF, Simon SD, Sharma V. Variation and Trends in ED Use of Radiographs for Asthma, Bronchiolitis, and Croup in Children. Pediatrics 2013; 132(2): 245-52.

404. Constant C, Magalhães M, Andrade MG, et al. Perspectives of pediatricians and general practitioners on diagnosis and treatment of bronchiolitis. Eur Respir J 2014; 44(Suppl 58).

405. Florin TA, Byczkowski T, Ruddy RM, Zorc JJ, Test M, Shah SS. Variation in the management of infants hospitalized for bronchiolitis persists after the 2006 American Academy of Pediatrics bronchiolitis guidelines. J Pediatr 2014; 165(4): 786-92 e1.

406. Librizzi J, McCulloh R, Koehn K, Alverson B. Appropriateness of Testing for Serious Bacterial Infection in Children Hospitalized With Bronchiolitis. Hospital Pediatrics 2014; 4(1): 33-8.

407. McCulloh RJ, Smitherman SE, Koehn KL, Alverson BK. Assessing the impact of national guidelines on the management of children hospitalized for acute bronchiolitis. Pediatr Pulmonol 2014; 49(7): 688-94.

408. Andrade MA, Hoberman A, Glustein J, Paradise JL, Wald ER. Acute Otitis Media in Children With Bronchiolitis. Pediatrics 1998; 101(4): 617-9.

409. Shazberg G, Revel-Vilk S, Shoseyov D, Ben-Ami A, Klar A, Hurvitz H. The clinical course of bronchiolitis associated with acute otitis media. Arch Dis Child 2000; 83(4): 317-9.

410. Gomaa MA, Galal O, Mahmoud MS. Risk of acute otitis media in relation to acute bronchiolitis in children. Int J Pediatr Otorhinolaryngol 2012; 76(1): 49-51.

411. Saijo M, Ishii T, Kokubo M, Murono K, Takimoto M, Fujita K. White blood cell count, C-reactive protein and erythrocyte sedimentation rate in respiratory syncytial virus infection of the lower respiratory tract. Acta Paediatr Jpn 1996; 38(6): 596-600.

412. Fares M, Mourad S, Rajab M, Rifai N. The use of C-reactive protein in predicting bacterial co-Infection in children with bronchiolitis. N Am J Med Sci 2011; 3(3): 152-6.

413. Laham JL, Breheny PJ, Gardner BM, Bada H. Procalcitonin to predict bacterial coinfection in infants with acute bronchiolitis: a preliminary analysis. Pediatr Emerg Care 2014; 30(1): 11-5.

414. Resch B, Gusenleitner W, Muller W. Procalcitonin, interleukin-6, C-reactive protein and leukocyte counts in infants with bronchiolitis. Pediatr Infect Dis J 2003; 22(5): 475-6.

415. Purcell K, Fergie J. Lack of Usefulness of an Abnormal White Blood Cell Count for Predicting a Concurrent Serious Bacterial Infection in Infants and Young Children Hospitalized With Respiratory Syncytial Virus Lower Respiratory Tract Infection. Pediatr Infect Dis J 2007; 26(4).

416. Costa S, Rocha R, Tavares M, Bonito-Vitor A, Guedes-Vaz L. C Reactive protein and disease severity in bronchiolitis. Rev Port Pneumol 2009; 15(1): 55-65.

417. Gouveia R, Saianda A, Vieira M, Mendes L, Nunes T, Lobo L, Bandeira T. Bronquiolite aguda. Poucas indicações para a utilização de exames complementares de diagnóstico. Acta Pediatr Port 2010;41(2):69-74.

418. Papoff P, Moretti C, Cangiano G, et al. Incidence and predisposing factors for severe disease in previously healthy term infants experiencing their first episode of bronchiolitis. Acta Paediatr 2011; 100(7): e17-23.

419. van der Meer V, Neven AK, van den Broek PJ, Assendelft WJ. Diagnostic value of C reactive protein in infections of the lower respiratory tract: systematic review. BMJ 2005; 331(7507): 26.

420. Flood RG, Badik J, Aronoff SC. The Utility of Serum C-Reactive Protein in Differentiating Bacterial from Nonbacterial Pneumonia in Children: A Meta-Analysis of 1230 Children. Pediatr Infect Dis J 2008; 27(2).

421. Sanders S, Barnett A, Correa-Velez I, Coulthard M, Doust J. Systematic Review of the Diagnostic Accuracy of C-Reactive Protein to Detect Bacterial Infection in Nonhospitalized Infants and Children with Fever. J Pediatr 2008; 153(4): 570-4.e3.

422. Van den Bruel A, Thompson MJ, Haj-Hassan T, et al. Diagnostic value of laboratory tests in identifying serious infections in febrile children: systematic review. BMJ 2011; 342: d3082.

423. Schuh S, Lalani A, Allen U, et al. Evaluation of the utility of radiography in acute bronchiolitis. J Pediatr 2007; 150(4): 429-33.

424. Kneyber MC, Moons KG, de Groot R, Moll HA. Predictors of a normal chest x-ray in respiratory syncytial virus infection. Pediatr Pulmonol 2001; 31(4): 277-83.

425. Garcia Garcia ML, Calvo Rey C, Quevedo Teruel S, et al. [Chest radiograph in bronchiolitis: is it always necessary?]. An Pediatr (Barc) 2004; 61(3): 219-25.

426. Ecochard-Dugelay E, Beliah M, Perreaux F, et al. Clinical predictors of radiographic abnormalities among infants with bronchiolitis in a paediatric emergency department. BMC Pediatr 2014; 14: 143.

427. Mahabee-Gittens EM, Dowd MD, Beck JA, Smith SZ. Clinical Factors Associated with Focal Infiltrates in Wheezing Infants and Toddlers. Clin Pediatr (Phila) 2000; 39(7): 387-93.

428. Davies HD, Wang EE, Manson D, Babyn P, Shuckett B. Reliability of the chest radiograph in the diagnosis of lower respiratory infections in young children. Pediatr Infect Dis J 1996; 15(7): 600-4.

429. Swingler GH. Observer variation in chest radiography of acute lower respiratory infections in children: a systematic review. BMC Med Imaging 2001; 1(1): 1.

430. Sarria E, Fischer GB, Lima JA, Menna Barreto SS, Flores JA, Sukiennik R. [Interobserver agreement in the radiological diagnosis of lower respiratory tract infections in children]. J Pediatr (Rio J) 2003; 79(6): 497-503.

431. Bada C, Carreazo NY, Chalco JP, Huicho L. Inter-observer agreement in interpreting chest X-rays on children with acute lower respiratory tract infections and concurrent wheezing. Sao Paulo Med J 2007; 125(3): 150-4.

432. Levinsky Y, Mimouni FB, Fisher D, Ehrlichman M. Chest radiography of acute paediatric lower respiratory infections: experience versus interobserver variation. Acta Paediatr 2013; 102(7): e310-4.

433. Xavier-Souza G, Vilas-Boas AL, Fontoura MS, et al. The inter-observer variation of chest radiograph reading in acute lower respiratory tract infection among children. Pediatr Pulmonol 2013; 48(5): 464-9.

434. Ecochard-Dugelay E, Beliah M, Boisson C, et al. Impact of Chest Radiography for Children with Lower Respiratory Tract Infection: A Propensity Score Approach. PLoS ONE 2014; 9(5): e96189.

435. Swingler GH, Zwarenstein M. WITHDRAWN: Chest radiograph in acute respiratory infections. Cochrane Database Syst Rev 2009; (4): Cd001268.

436. Carsin A, Gorincour G, Bresson V, et al. La radiographie de thorax chez le nourrisson hospitalisé pour bronchiolite aiguë : réelle information ou simple irradiation ? Arch Pediatr 2012; 19(12): 1308-15.

437. Harris C, Turner T, Mazza D, Wilkinson F, Health for Kids Guideline Development G. Guideline development process for the Health for Kids in the South East project. Aust Fam Physician 2008; 37(6): 2-5.

438. Krasinski K, LaCouture R, Holzman RS, Waithe E, Bonk S, Hanna B. Screening for respiratory syncytial virus and assignment to a cohort at admission to reduce nosocomial transmission. J Pediatr 1990; 116(6): 894-8.

439. Isaacs D, Dickson H, O'Callaghan C, Sheaves R, Winter A, Moxon ER. Handwashing and cohorting in prevention of hospital acquired infections with respiratory syncytial virus. Arch Dis Child 1991; 66(2): 227-31.

440. Simon A, Khurana K, Wilkesmann A, et al. Nosocomial respiratory syncytial virus infection: impact of prospective surveillance and targeted infection control. Int J Hyg Environ Health 2006; 209(4): 317-24.

441. Siegel JD, Rhinehart E, Jackson M, Chiarello L, Health Care Infection Control Practices Advisory C. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Health Care Settings. Am J Infect Control 2007; 35(10 Suppl 2): S65-164.

442. Seto WH, Conly JM, Pessoa-Silva CL, Malik M, Eremin S. Infection prevention and control measures for acute respiratory infections in healthcare settings: an update. East Mediterr Health J 2013; 19 Suppl 1: S39-47.

443. Karanfil LV, Conlon M, Lykens K, et al. Reducing the rate of nosocomially transmitted respiratory syncytial virus. Am J Infect Control 1999; 27(2): 91-6.

444. Bekhof J, Bakker J, Reimink R, et al. Co-infections in children hospitalised for bronchiolitis: role of roomsharing. J Clin Med Res 2013; 5(6): 426-31..

445. Thorburn K, Hart CA. Think outside the box: extrapulmonary manifestations of severe respiratory syncytial virus infection. Crit Care 2006; 10(4): 159.

446. Hanna S, Tibby SM, Durward A, Murdoch IA. Incidence of hyponatraemia and hyponatraemic seizures in severe respiratory syncytial virus bronchiolitis. Acta Paediatr 2003; 92(4): 430-4.

447. van Steensel-Moll HA, Hazelzet JA, van der Voort E, Neijens HJ, Hackeng WH. Excessive secretion of antidiuretic hormone in infections with respiratory syncytial virus. Arch Dis Child 1990; 65(11): 1237-9.

448. Foster BA, Tom D, Hill V. Hypotonic versus Isotonic Fluids in Hospitalized Children: A Systematic Review and Meta-Analysis. J Pediatr 2014; 165(1): 163-9.e2.

449. Mansbach JM, Clark S, Christopher NC, et al. Prospective Multicenter Study of Bronchiolitis: Predicting Safe Discharges From the Emergency Department. Pediatrics 2008; 121(4): 680-8.

450. Swingler GH, Hussey GD, Zwarenstein M. DUration of illness in ambulatory children diagnosed with bronchiolitis. Arch Pediatr Adolesc Med 2000; 154(10): 997-1000.

451. Robbins JM, Kotagal UR, Kini NM, Mason WH, Parker JG, Kirschbaum MS. At-home recovery following hospitalization for bronchiolitis. Ambul Pediatr 2006; 6(1): 8-14.

452. Petruzella FD, Gorelick MH. Duration of Illness in Infants With Bronchiolitis Evaluated in the Emergency Department. Pediatrics 2010; 126(2): 285-90.

453. Norwood A, Mansbach JM, Clark S, Waseem M, Camargo CA. Prospective Multicenter Study of Bronchiolitis: Predictors of an Unscheduled Visit After Discharge From the Emergency Department. Acad Emerg Med 2010; 17(4): 376-82.

454. Wittig HJ, Glaser J. The relationship between bronchiolitis and childhood asthma; a follow-up study of 100 cases of bronchiolitis. J Allergy 1959; 30(1): 19-23.

455. Zweiman B, Schoenwetter WF, Hildreth EA. The relationship between bronchiolitis and allergic asthma: A prospective study with allergy evaluation. J Allergy 1966; 37(1): 48-53.

456. Rooney JC, Williams HE. The relationship between proved viral bronchiolitis and subsequent wheezing. J Pediatr 1971; 79(5): 744-7.

457. McConnochie KM, Roghmann KJ, Klein SJ, et al. Bronchiolitis as a Possible Cause of Wheezing in Childhood: New Evidence. Pediatrics 1984; 74(1): 1-10.

458. Martinez FD. Heterogeneity of the association between lower respiratory illness in infancy and subsequent asthma. Proc Am Thorac Soc 2005; 2(2): 157-61.

459. Stein RT, Martinez FD. Respiratory syncytial virus and asthma: still no final answer. Thorax 2010; 65(12): 1033-4.

460. Brand PLP, Baraldi E, Bisgaard H, et al. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. Eur Respir J 2008; 32(4): 1096-110.

461. Saglani S, Bush A. Asthma in preschool children: the next challenge. Curr Opin Allergy Clin Immunol 2009; 9(2): 141-5.

462. Spycher BD, Silverman M, Kuehni CE. Phenotypes of childhood asthma: are they real? Clin Exp Allergy 2010; 40(8): 1130-41.

463. Cowan K, Guilbert TW. Pediatric asthma phenotypes. Curr Opin Pediatr 2012; 24(3): 344-51.

464. Brand PLP, Caudri D, Eber E, et al. Classification and pharmacological treatment of preschool wheezing: changes since 2008. Eur Respir J 2014; 43(4): 1172-7.

465. Ducharme FM, Tse SM, Chauhan B. Diagnosis, management, and prognosis of preschool wheeze. Lancet 2014; 383(9928): 1593-604.

466. Kneyber MCJ, Steyerberg EW, de Groot R, Moll HA. Long-term effects of respiratory syncytial virus (RSV) bronchiolitis in infants and young children: a quantitative review. Acta Paediatr 2000; 89(6): 654-60.

467. Regnier SA, Huels J. Association between respiratory syncytial virus hospitalizations in infants and respiratory sequelae: systematic review and meta-analysis. Pediatr Infect Dis J 2013; 32(8): 820-6.

468. Szabo SM, Levy AR, Gooch KL, Bradt P, Wijaya H, Mitchell I. Elevated risk of asthma after hospitalization for respiratory syncytial virus infection in infancy. Paediatr Respir Rev 2013; 13 Suppl 2: S9-15.

469. Thomsen SF, Stensballe LG, Skytthe A, Kyvik KO, Backer V, Bisgaard H. Increased Concordance of Severe Respiratory Syncytial Virus Infection in Identical Twins. Pediatrics 2008; 121(3): 493-6.

470. Stensballe LG, Simonsen JB, Thomsen SF, et al. The causal direction in the association between respiratory syncytial virus hospitalization and asthma. J Allergy Clin Immunol 2009; 123(1): 131-7.e1.

471. Thomsen SF, van der Sluis S, Stensballe LG, et al. Exploring the Association between Severe Respiratory Syncytial Virus Infection and Asthma. Am J Respir Crit Care Med 2009; 179(12): 1091-7.

472. Poorisrisak P, Halkjaer LB, Thomsen SF, et al. CAusal direction between respiratory syncytial virus bronchiolitis and asthma studied in monozygotic twins. Chest 2010; 138(2): 338-44.

473. Ermers MJJ, Hoebee B, Hodemaekers HM, Kimman TG, Kimpen JLL, Bont L. IL-13 genetic polymorphism identifies children with late wheezing after respiratory syncytial virus infection. J Allergy Clin Immunol 2007; 119(5): 1086-91.

474. Ermers MJJ, Janssen R, Onland-Moret NC, et al. IL10 Family Member Genes IL19 and IL20 Are Associated With Recurrent Wheeze After Respiratory Syncytial Virus Bronchiolitis. Pediatr Res 2011; 70(5): 518-23.

475. Drysdale SB, Wilson T, Alcazar M, et al. Lung function prior to viral lower respiratory tract infections in prematurely born infants. Thorax 2011; 66(6): 468-73.

476. Zomer-Kooijker K, Uiterwaal CS, van der Gugten AC, Wilbrink B, Bont LJ, van der Ent CK. Decreased lung function precedes severe respiratory syncytial virus infection and post-respiratory syncytial virus wheeze in term infants. Eur Respir J 2014; 44(3): 666-74.

477. van der Zalm MM, Uiterwaal CSPM, Wilbrink B, Koopman M, Verheij TJM, van der Ent CK. The Influence of Neonatal Lung Function on Rhinovirus-associated Wheeze. Am J Respir Crit Care Med 2011; 183(2): 262-7.

478. Chawes BL, Poorisrisak P, Johnston SL, Bisgaard H. Neonatal bronchial hyperresponsiveness precedes acute severe viral bronchiolitis in infants. J Allergy Clin Immunol 2012; 130(2): 354-61.e3.

479. Young S, O'Keeffe PT, Arnott J, Landau LI. Lung function, airway responsiveness, and respiratory symptoms before and after bronchiolitis. Arch Dis Child 1995; 72(1): 16-24.

480. Tager IB, Hanrahan JP, Tosteson TD, et al. Lung function, pre- and post-natal smoke exposure, and wheezing in the first year of life. Am Rev Respir Dis 1993; 147(4): 811-7.

481. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med 1995; 332(3): 133-8.

482. Dezateux C, Stocks J, Dundas I, Fletcher ME. Impaired airway function and wheezing in infancy: the influence of maternal smoking and a genetic predisposition to asthma. Am J Respir Crit Care Med 1999; 159(2): 403-10.

483. Murray CS, Pipis SD, McArdle EC, Lowe LA, Custovic A, Woodcock A. Lung function at one month of age as a risk factor for infant respiratory symptoms in a high risk population. Thorax 2002; 57(5): 388-92.

484. Turner SW, Palmer LJ, Rye PJ, et al. Infants with flow limitation at 4 weeks: outcome at 6 and 11 years. Am J Respir Crit Care Med 2002; 165(9): 1294-8.

485. Turner SW, Young S, Landau LI, Le Souëf PN. Reduced lung function both before bronchiolitis and at 11 years. Arch Dis Child 2002; 87(5): 417-20.

486. Haland G, Carlsen KC, Sandvik L, et al. Reduced lung function at birth and the risk of asthma at 10 years of age. N Engl J Med 2006; 355(16): 1682-9.

487. Martinez FD. The origins of asthma and chronic obstructive pulmonary disease in early life. Proc Am Thorac Soc 2009; 6(3): 272-7.

488. Pike KC, Rose-Zerilli MJ, Osvald EC, et al. The relationship between infant lung function and the risk of wheeze in the preschool years. Pediatr Pulmonol 2011; 46(1): 75-82.

489. Stern DA, Morgan WJ, Wright AL, Guerra S, Martinez FD. Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study. Lancet 2007; 370(9589): 758-64.

490. Mullane D, Turner SW, Cox DW, Goldblatt J, Landau LI, le Souëf PN. REduced infant lung function, active smoking, and wheeze in 18-year-old individuals. JAMA Pediatrics 2013; 167(4): 368-73.

491. Turner S, Fielding S, Mullane D, et al. A longitudinal study of lung function from 1 month to 18 years of age. Thorax 2014; 69(11): 1015-20.

492. Broughton S, Roberts A, Fox G, et al. Prospective study of healthcare utilisation and respiratory morbidity due to RSV infection in prematurely born infants. Thorax 2005; 60(12): 1039-44.

493. Hennessy EM, Bracewell MA, Wood N, et al. Respiratory health in pre-school and school age children following extremely preterm birth. Arch Dis Child 2008; 93(12): 1037-43.

494. Fawke J, Lum S, Kirkby J, et al. Lung Function and Respiratory Symptoms at 11 Years in Children Born Extremely Preterm. Am J Respir Crit Care Med 2010; 182(2): 237-45.

495. Montgomery S, Bahmanyar S, Brus O, Hussein O, Kosma P, Palme-Kilander C. Respiratory infections in preterm infants and subsequent asthma: a cohort study. BMJ Open 2013; 3(10).

496. Gern JE, Brooks GD, Meyer P, et al. Bidirectional interactions between viral respiratory illnesses and cytokine responses in the first year of life. J Allergy Clin Immunol 2006; 117(1): 72-8.

497. Fjaerli H-O, Bukholm G, Skjaeret C, Holden M, Nakstad B. Cord Blood Gene Expression in Infants Hospitalized with Respiratory Syncytial Virus Bronchiolitis. J Infect Dis 2007; 196(3): 394-404.

498. Stern DA, Guerra S, Halonen M, Wright AL, Martinez FD. Low IFN-gamma production in the first year of life as a predictor of wheeze during childhood. J Allergy Clin Immunol 2007; 120(4): 835-41.

499. Carroll KN, Wu P, Gebretsadik T, et al. The severity-dependent relationship of infant bronchiolitis on the risk and morbidity of early childhood asthma. J Allergy Clin Immunol 2009; 123(5): 1055-61.e1.

500. Simoes EAF, Groothuis JR, Carbonell-Estrany X, et al. Palivizumab Prophylaxis, Respiratory Syncytial Virus, and Subsequent Recurrent Wheezing. J Pediatr 2007; 151(1): 34-42.e1.

501. Simoes EA, Carbonell-Estrany X, Rieger CH, Mitchell I, Fredrick L, Groothuis JR. The effect of respiratory syncytial virus on subsequent recurrent wheezing in atopic and nonatopic children. J Allergy Clin Immunol 2010; 126(2): 256-62.

502. Krishnamoorthy N, Khare A, Oriss TB, et al. Early infection with respiratory syncytial virus impairs regulatory T cell function and increases susceptibility to allergic asthma. Nat Med 2012; 18(10): 1525-30.

503. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B, Björkstén B. Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. Pediatrics 1995; 95(4): 500-5.

504. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. Am J Respir Crit Care Med 2000; 161(5): 1501-7.

505. Sigurs N. A cohort of children hospitalised with acute RSV bronchiolitis: impact on later respiratory disease. Paediatr Respir Rev 2002; 3(3): 177-83.

506. Sigurs N, Gustafsson PM, Bjarnason R, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. Am J Respir Crit Care Med 2005; 171(2): 137-41.

507. Sigurs N. Respiratory syncytial virus lower respiratory tract illness in infancy and subsequent morbidity. Acta Paediatr 2007; 96(2): 156-7.

508. Sigurs N, Aljassim F, Kjellman B, et al. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. Thorax 2010; 65(12): 1045-52.

509. Lukkarinen M, Lukkarinen H, Lehtinen P, Vuorinen T, Ruuskanen O, Jartti T. Prednisolone reduces recurrent wheezing after first rhinovirus wheeze: a 7-year follow-up. Pediatr Allergy Immunol 2013; 24(3): 237-43.

510. Kotaniemi-Syrjänen A, Vainionpää R, Reijonen TM, Waris M, Korhonen K, Korppi M. Rhinovirus-induced wheezing in infancy—the first sign of childhood asthma? J Allergy Clin Immunol 2003; 111(1): 66-71.

511. Hyvarinen MK, Kotaniemi-Syrjanen A, Reijonen TM, Korhonen K, Korppi MO. Teenage asthma after severe early childhood wheezing: an 11-year prospective follow-up. Pediatr Pulmonol 2005; 40(4): 316-23.

512. Jackson DJ, Gangnon RE, Evans MD, et al. Wheezing Rhinovirus Illnesses in Early Life Predict Asthma Development in High-Risk Children. Am J Respir Crit Care Med 2008; 178(7): 667-72.

513. Jartti T, Korppi M. Rhinovirus-induced bronchiolitis and asthma development. Pediatr Allergy Immunol 2011; 22(4): 350-5.

514. Ruotsalainen M, Hyvarinen MK, Piippo-Savolainen E, Korppi M. Adolescent asthma after rhinovirus and respiratory syncytial virus bronchiolitis. Pediatr Pulmonol 2013; 48(7): 633-9.

515. Korppi M, Kotaniemi-Syrjänen A, Waris M, Vainionpää R, Reijonen TM. Rhinovirus-associated wheezing in infancy: comparison with respiratory syncytial virus bronchiolitis. Pediatr Infect Dis J 2004; 23(11): 995-9.

516. Henderson J, Granell R, Sterne J. The search for new asthma phenotypes. Arch Dis Child 2009.

517. Martinez FD, Vercelli D. Asthma. Lancet 2013; 382(9901): 1360-72.

518. Martinez FD. Genes, environments, development and asthma: a reappraisal. Eur Respir J 2007; 29(1): 179-84.

519. Holt PG, Sly PD. Viral infections and atopy in asthma pathogenesis: new rationales for asthma prevention and treatment. Nat Med 2012; 18(5): 726-35.

520. Saglani S, Malmstrom K, Pelkonen AS, et al. Airway remodeling and inflammation in symptomatic infants with reversible airflow obstruction. Am J Respir Crit Care Med 2005; 171(7): 722-7.

521. Saglani S, Payne DN, Zhu J, et al. Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. Am J Respir Crit Care Med 2007; 176(9): 858-64.

522. Bush A. How early do airway inflammation and remodeling occur? Allergol Int 2008; 57(1): 11-9.

523. O'Reilly R, Ullmann N, Irving S, et al. Increased airway smooth muscle in preschool wheezers who have asthma at school age. J Allergy Clin Immunol 2013; 131(4): 1024-32, 32.e1-16.

524. Bouzigon E, Corda E, Aschard H, et al. Effect of 17q21 variants and smoking exposure in early-onset asthma. N Engl J Med 2008; 359(19): 1985-94.

525. van der Valk RJ, Duijts L, Kerkhof M, et al. Interaction of a 17q12 variant with both fetal and infant smoke exposure in the development of childhood asthma-like symptoms. Allergy 2012; 67(6): 767-74.

526. Caliskan M, Bochkov YA, Kreiner-Moller E, et al. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. N Engl J Med 2013; 368(15): 1398-407.

527. Savenije OE, Kerkhof M, Koppelman GH, Postma DS. Predicting who will have asthma at school age among preschool children. J Allergy Clin Immunol 2012; 130(2): 325-31.

528. Blom D, Ermers M, Bont L, van Aalderen WM, van Woensel JB. Inhaled corticosteroids during acute bronchiolitis in the prevention of post-bronchiolitic wheezing. Cochrane Database Syst Rev 2007; (1): Cd004881.

529. Ermers MJ, Rovers MM, van Woensel JB, Kimpen JL, Bont LJ. The effect of high dose inhaled corticosteroids on wheeze in infants after respiratory syncytial virus infection: randomised double blind placebo controlled trial. BMJ 2009; 338: b897.

530. Zomer-Kooijker K, van der Ent CK, Ermers MJ, Rovers MM, Bont LJ. Lack of long-term effects of high-dose inhaled beclomethasone for respiratory syncytial virus bronchiolitis: a randomized placebo-controlled trial. Pediatr Infect Dis J 2014; 33(1): 19-23.

531. Bush A. COPD: a pediatric disease. COPD 2008; 5(1): 53-67.

532. Bandeira T. Contribuição para o estudo das origens pediátricas da doença pulmonar obstrutiva crónica do adulto: bronquiolite obliterante pós-infecciosa [PhD]. Lisboa: Universidade de Lisboa, Faculdade de Medicina; 2010.

533. Narang I, Bush A. Early origins of chronic obstructive pulmonary disease. Semin Fetal Neonatal Med 2012; 17(2): 112-8.

534. Kerkhof M, Boezen HM, Granell R, et al. Transient early wheeze and lung function in early childhood associated with chronic obstructive pulmonary disease genes. J Allergy Clin Immunol 2014; 133(1): 68-76 e1-4.

535. Barker DJ, Osmond C, Law CM. The intrauterine and early postnatal origins of cardiovascular disease and chronic bronchitis. J Epidemiol Community Health 1989; 43(3): 237-40.

536. Barker DJ. The fetal and infant origins of adult disease. BMJ 1990; 301(6761): 1111.

537. Lucas A, Fewtrell MS, Cole TJ. Fetal origins of adult disease-the hypothesis revisited. BMJ 1999; 319(7204): 245-9.

538. Postma DS, Kerkhof M, Boezen HM, Koppelman GH. Asthma and chronic obstructive pulmonary disease: common genes, common environments? Am J Respir Crit Care Med 2011; 183(12): 1588-94.

539. Kimpen JL, Schaad UB. Treatment of respiratory syncytial virus bronchiolitis: 1995 poll of members of the European Society for Paediatric Infectious Diseases. Pediatr Infect Dis J 1997; 16(5): 479-81.

540. Kornecki A, Shemie SD. Bronchodilators and RSV-induced respiratory failure: agonizing about beta2 agonists. Pediatr Pulmonol. United states; 1998: 4-5.

541. Scarfone RJ. Controversies in the treatment of bronchiolitis. Curr Opin Pediatr 2005; 17(1): 62-6.

542. Peng WS, Chen X, Yang XY, Liu EM. Systematic review of montelukast's efficacy for preventing post-bronchiolitis wheezing. Pediatr Allergy Immunol 2014; 25(2): 143-50.

543. Phelan PD, Stocks JG. Management of severe viral bronchiolitis and severe acute asthma. Arch Dis Child 1974; 49(2): 143-8.

544. Schepens B, Ibañez LI, De Baets S, et al. Nanobodies® Specific for Respiratory Syncytial Virus Fusion Protein Protect Against Infection by Inhibition of Fusion. J Infect Dis 2011; 204(11): 1692-701.

545. Devincenzo J, Fathi H, Mcclure M, et al. LATE BREAKER ABSTRACTS. Open Forum Infectious Diseases 2014; 1(suppl 1): S66-S9.

546. Murray J, Saxena S, Sharland M. Preventing severe respiratory syncytial virus disease: passive, active immunisation and new antivirals. Arch Dis Child 2014;99(5):469-73

547. Johnson LW, Robles J, Hudgins A, Osburn S, Martin D, Thompson A. Management of Bronchiolitis in the Emergency Department: Impact of Evidence-Based Guidelines? Pediatrics 2013; 131(Supplement 1): S103-S9.

548. Behrendt CE, Decker MD, Burch DJ, Watson PH. International variation in the management of infants hospitalized with respiratory syncytial virus. International RSV Study Group. Eur J Pediatr 1998; 157(3): 215-20.

549. Brand PL, Vaessen-Verberne AA. Differences in management of bronchiolitis between hospitals in The Netherlands. Dutch Paediatric Respiratory Society. Eur J Pediatr 2000; 159(5): 343-7.

550. Offer I, Ashkenazi S, Livni G, Shalit I. The diagnostic and therapeutic approach to acute bronchiolitis in hospitalized children in Israel: a nationwide survey. Isr Med Assoc J 2000; 2(2): 108-10.

551. Barben J, Kuehni CE, Trachsel D, Hammer J. Management of acute bronchiolitis: can evidence based guidelines alter clinical practice? Thorax 2008; 63(12): 1103-9.

552. David M, Luc-Vanuxem C, Loundou A, Bosdure E, Auquier P, Dubus JC. [Assessment of the French Consensus Conference for Acute Viral Bronchiolitis on outpatient management: progress between 2003 and 2008]. Arch Pediatr 2010; 17(2): 125-31.

553. Ochoa Sangrador C, Gonzalez de Dios J. Management of acute bronchiolitis in emergency wards in Spain: variability and appropriateness analysis (aBREVIADo Project). Eur J Pediatr 2012; 171(7): 1109-19.

554. Mecklin M, Hesselmar B, Qvist E, Wennergren G, Korppi M. Diagnosis and treatment of bronchiolitis in Finnish and Swedish children's hospitals. Acta Paediatr 2014.

555. Ochoa Sangrador C, Gonzalez de Dios J. Overuse of bronchodilators and steroids in bronchiolitis of different severity: Bronchiolitis-study of variability, appropriateness, and adequacy. Allergol Immunopathol (Madr) 2014; 42(4): 307-15.

556. Florin TA, Byczkowski T, Ruddy RM, Zorc JJ, Test M, Shah SS. Variation in the management of infants hospitalized for bronchiolitis persists after the 2006 American Academy of Pediatrics bronchiolitis guidelines. J Pediatr 2014; 165(4): 786-92 e1.

557. Christakis DA, Cowan CA, Garrison MM, Molteni R, Marcuse E, Zerr DM. Variation in Inpatient Diagnostic Testing and Management of Bronchiolitis. Pediatrics 2005; 115(4): 878-84.

558. Conway PH, Edwards S, Stucky ER, Chiang VW, Ottolini MC, Landrigan CP. Variations in Management of Common Inpatient Pediatric Illnesses: Hospitalists and Community Pediatricians. Pediatrics 2006; 118(2): 441-7.

559. Fernanda Rodrigues, Manuela Costa Alves, Luís Lemos. Bronquiolites em Unidade de Internamento de Curta Duração: a orientação terapêutica na prática clínica. Acta Pediatr Port. 2007;38(5):183-6

560. De Brasi D, Pannuti F, Antonelli F, de Seta F, Siani P, de Seta L. Therapeutic approach to bronchiolitis: why pediatricians continue to overprescribe drugs? Ital J Pediatr 2010; 36: 67.

561. Maxwell S. Rational prescribing: the principles of drug selection. Clin Med 2009; 9(5): 481-5.

562. World Health O. The rational use of drugs. Report of the Conference of Experts. Geneva: WHO; 1985.

563. Goodman DC. Unwarranted variation in pediatric medical care. Pediatr Clin North Am 2009; 56(4): 745-55.

564. Davis D, Evans M, Jadad A, et al. The case for knowledge translation: shortening the journey from evidence to effect. BMJ 2003; 327(7405): 33-5.

565. Grimshaw JM, Santesso N, Cumpston M, Mayhew A, McGowan J. Knowledge for knowledge translation: the role of the Cochrane Collaboration. J Contin Educ Health Prof 2006; 26(1): 55-62.

566. Straus SE, Graham ID, Mazmanian PE. Knowledge translation: resolving the confusion. J Contin Educ Health Prof 2006; 26(1): 3-4.

567. Ralston S, Garber M, Narang S, et al. Decreasing unnecessary utilization in acute bronchiolitis care: results from the value in inpatient pediatrics network. J Hosp Med 2013; 8(1): 25-30.

568. Akenroye AT, Baskin MN, Samnaliev M, Stack AM. Impact of a bronchiolitis guideline on ED resource use and cost: a segmented time-series analysis. Pediatrics 2014; 133(1): e227-34.

569. Mittal V, Hall M, Morse R, et al. Impact of inpatient bronchiolitis clinical practice guideline implementation on testing and treatment. J Pediatr 2014; 165(3): 570-6 e3.

570. Mittal V, Darnell C, Walsh B, et al. Inpatient bronchiolitis guideline implementation and resource utilization. Pediatrics 2014; 133(3): e730-7.

571. Parikh K, Hall M, Teach SJ. Bronchiolitis management before and after the AAP guidelines. Pediatrics 2014; 133(1): e1-7.

572. Ralston S, Comick A, Nichols E, Parker D, Lanter P. Effectiveness of quality improvement in hospitalization for bronchiolitis: a systematic review. Pediatrics 2014; 134(3): 571-81.

573. GW TJ, Eland IA, Sturkenboom MC, van den Anker JN, Stricker BH. Unlicensed and off label prescription of drugs to children: population based cohort study. BMJ 2002; 324(7349): 1313-4.

574. Klassen TP, Hartling L, Craig JC, Offringa M. Children are not just small adults: the urgent need for high-quality trial evidence in children. PLoS Med 2008; 5(8): e172.

575. Frattarelli DA, Galinkin JL, Green TP, et al. Off-label use of drugs in children. Pediatrics 2014; 133(3): 563-7.

576. Turner MA, Catapano M, Hirschfeld S, Giaquinto C, Global Research in P. Paediatric drug development: the impact of evolving regulations. Adv Drug Deliv Rev 2014; 73: 2-13.

577. Fouretier A, Bertram D. New regulations on medical devices in Europe: what to expect? Expert Rev Med Devices 2014; 11(4): 351-9.

578. Leer JA, Jr., Green JL, Heimlich EM, et al. Corticosteroid treatment in bronchiolitis. A controlled, collaborative study in 297 infants and children. Am J Dis Child (1960) 1969; 117(5): 495-503.

579. de Benedictis FM, Bush A. Corticosteroids in respiratory diseases in children. Am J Respir Crit Care Med 2012; 185(1): 12-23.

580. Szefler SJ, Chmiel JF, Fitzpatrick AM, et al. Asthma across the ages: knowledge gaps in childhood asthma. J Allergy Clin Immunol 2014; 133(1): 3-13; quiz 4.

581. Amirav I, Newhouse MT. Aerosol therapy in infants and toddlers: past, present and future. Expert Rev Respir Med 2008; 2(5): 597-605.

582. Smyth AR, Barbato A, Beydon N, et al. Respiratory medicines for children: current evidence, unlicensed use and research priorities. Eur Respir J 2010; 35(2): 247-65.

583. Oxman AD, Sackett DL, Guyatt GH. Users' guides to the medical literature. I. How to get started. The Evidence-Based Medicine Working Group. J Am Med Assoc 1993; 270(17): 2093-5.

584. Glasziou P, Ogrinc G, Goodman S. Can evidence-based medicine and clinical quality improvement learn from each other? BMJ Qual Saf 2011; 20 Suppl 1: i13-7.

585. Connolly JH, Field CM, Glasgow JF, Slattery CM, MacLynn DM. A double blind trial of prednisolone in epidemic bronchiolitis due to respiratory syncytial virus. Acta Paediatr Scand 1969; 58(2): 116-20.

586. Stokes GM, Milner AD, Hodges IG, Henry RL, Elphick MC. Nebulised therapy in acute severe bronchiolitis in infancy. Arch Dis Child 1983; 58(4): 279-82.

587. Garrison MM, Christakis DA, Harvey E, Cummings P, Davis RL. Systemic corticosteroids in infant bronchiolitis: A meta-analysis. Pediatrics 2000; 105(4): E44.

588. DiTraglia J. Bronchiolitis. Arch Pediatr Adolesc Med. United States; 2004: 707; author reply -8.

589. King VJ, Viswanathan M, Bordley WC, et al. Pharmacologic treatment of bronchiolitis in infants and children: a systematic review. Arch Pediatr Adolesc Med 2004; 158(2): 127-37.

590. Weinberger M. Should corticosteroids be used for first-time young wheezers? J Allergy Clin Immunol. United States; 2007: 567-9.

591. Cazzola M, Page CP, Calzetta L, Matera MG. Pharmacology and therapeutics of bronchodilators. Pharmacol Rev 2012; 64(3): 450-504.

592. Proskocil BJ, Fryer AD. Beta2-agonist and anticholinergic drugs in the treatment of lung disease. Proc Am Thorac Soc 2005; 2(4): 305-10; discussion 11-2.

593. Barnes PJ, Basbaum CB, Nadel JA. Autoradiographic localization of autonomic receptors in airway smooth muscle. Marked differences between large and small airways. Am Rev Respir Dis 1983; 127(6): 758-62.

594. Mak JC, Barnes PJ. Autoradiographic visualization of muscarinic receptor subtypes in human and guinea pig lung. Am Rev Respir Dis 1990; 141(6): 1559-68.

595. Mak JC, Baraniuk JN, Barnes PJ. Localization of muscarinic receptor subtype mRNAs in human lung. Am J Respir Cell Mol Biol 1992; 7(3): 344-8.

596. Ikeda T, Anisuzzaman AS, Yoshiki H, et al. Regional quantification of muscarinic acetylcholine receptors and beta-adrenoceptors in human airways. Br J Pharmacol 2012; 166(6): 1804-14.

597. Carstairs JR, Nimmo AJ, Barnes PJ. Autoradiographic visualization of beta-adrenoceptor subtypes in human lung. Am Rev Respir Dis 1985; 132(3): 541-7.

598. Hall IP. Chapter 49 - β2-Adrenoceptor Agonists. In: Barnes PJ, Drazen JM, Rennard S, Thomson NC, eds. Asthma and COPD. London: Academic Press; 2002: 521-6.

599. Bassford CR, Thickett DR, Perkins GD. The rise and fall of beta-agonists in the treatment of ARDS. Crit Care 2012; 16(2): 208.

600. Aronson JK. "Where name and image meet"--the argument for "adrenaline". BMJ 2000; 320(7233): 506-9.

601. Zhang L, Sanguebsche LS. [The safety of nebulization with 3 to 5 ml of adrenaline (1:1000) in children: an evidence based review]. J Pediatr (Rio J) 2005; 81(3): 193-7.

602. Waisman Y, Klein BL, Boenning DA, et al. Prospective randomized double-blind study comparing L-epinephrine and racemic epinephrine aerosols in the treatment of laryngotracheitis (croup). Pediatrics 1992; 89(2): 302-6.

603. Bozzola E, Bozzola M, Barberi S, Cutrera R, Villani A. Safety and Potential Side Effects of β2-Agonists: A Still Debated Question. Int J Pediatr 2013; 1(1): 5.

604. Bonnelykke K, Jespersen JJ, Bisgaard H. Age dependent systemic exposure to inhaled salbutamol. British journal of clinical pharmacology 2007; 64(2): 241-4.

605. Bonnelykke K, Jespersen JJ, Bisgaard H. Early bioavailability of inhaled salbutamol reflects lung dose in children. British journal of clinical pharmacology. England; 2008: 562-3.

606. Auais A, Wedde-Beer K, Piedimonte G. Anti-inflammatory effect of albuterol enantiomers during respiratory syncytial virus infection in rats. Pediatr Pulmonol 2005; 40(3): 228-34.

607. Ameredes BT, Calhoun WJ. (R)-albuterol for asthma: pro [a.k.a. (S)-albuterol for asthma: con]. Am J Respir Crit Care Med. United States; 2006: 965-9; discussion 72-4.

608. Barnes PJ. Treatment with (R)-albuterol has no advantage over racemic albuterol. Am J Respir Crit Care Med. United States; 2006: 969-72; discussion 72-4.

609. Gosens R, Zaagsma J, Meurs H, Halayko AJ. Muscarinic receptor signaling in the pathophysiology of asthma and COPD. Respir Res 2006; 7: 73.

610. Piedimonte G. Contribution of neuroimmune mechanisms to airway inflammation and remodeling during and after respiratory syncytial virus infection. Pediatr Infect Dis J 2003; 22(2 Suppl): S66-74; discussion S-5.

611. Davis IC, Xu A, Gao Z, et al. Respiratory syncytial virus induces insensitivity to beta-adrenergic agonists in mouse lung epithelium in vivo. Am J Physiol Lung Cell Mol Physiol 2007; 293(2): L281-9.

612. Traylor ZP, Yu EN, Davis IC. Respiratory syncytial virus induces airway insensitivity to beta-agonists in BALB/c mice. Am J Physiol Lung Cell Mol Physiol 2010; 298(3): L437-45.

613. Trian T, Moir LM, Ge Q, et al. Rhinovirus-induced exacerbations of asthma: How is the {beta}2-adrenoceptor implicated? Am J Respir Cell Mol Biol 2010; 43(2): 227-33.

614. Alkhouri H, Rumzhum NN, Rahman MM, et al. TLR2 activation causes tachyphylaxis to beta2 -agonists in vitro and ex vivo: modelling bacterial exacerbation. Allergy 2014; 69(9): 1215-22.

615. Barr FE, Patel NR, Newth CJ. The pharmacologic mechanism by which inhaled epinephrine reduces airway obstruction in respiratory syncytial virus-associated bronchiolitis. J Pediatr 2000; 136(5): 699-700.

616. Prendiville A, Green S, Silverman M. Airway responsiveness in wheezy infants: evidence for functional beta adrenergic receptors. Thorax 1987; 42(2): 100-4.

617. Prendiville A, Green S, Silverman M. Ipratropium bromide and airways function in wheezy infants. Arch Dis Child 1987; 62(4): 397-400.

618. Hammer J, Numa A, Newth CJ. Albuterol responsiveness in infants with respiratory failure caused by respiratory syncytial virus infection. J Pediatr 1995; 127(3): 485-90.

619. Derish M, Hodge G, Dunn C, Ariagno R. Aerosolized albuterol improves airway reactivity in infants with acute respiratory failure from respiratory syncytial virus. Pediatr Pulmonol 1998; 26(1): 12-20.

620. Lodrup Carlsen KC, Carlsen KH. Inhaled nebulized adrenaline improves lung function in infants with acute bronchiolitis. Respir Med 2000; 94(7): 709-14.

621. Modl M, Eber E, Weinhandl E, Gruber W, Zach MS. Assessment of bronchodilator responsiveness in infants with bronchiolitis. A comparison of the tidal and the raised volume rapid thoracoabdominal compression technique. Am J Respir Crit Care Med 2000; 161(3 Pt 1): 763-8.

622. Sanchez I, Vizcaya C, Garcia D, Campos E. Response to bronchodilator in infants with bronchiolitis can be predicted from wheeze characteristics. Respirology 2005; 10(5): 603-8.

623. Carroll CL, Sala K, Zucker AR, Schramm CM. Pulmonary mechanics following albuterol therapy in mechanically ventilated infants with bronchiolitis. J Asthma 2012; 49(7): 688-96.

624. Prendiville A, Green S, Silverman M. Bronchial responsiveness to histamine in wheezy infants. Thorax 1987; 42(2): 92-9.

625. Silverman M, Prendiville A. Airway responsiveness in infancy. Am Rev Respir Dis 1987; 136(4 Pt 2): S71-3.

626. Henderson AJ, Young S, Stick SM, Landau LI, LeSouef PN. Effect of salbutamol on histamine induced bronchoconstriction in healthy infants. Thorax 1993; 48(4): 317-23.

627. Goldstein AB, Castile RG, Davis SD, et al. Bronchodilator responsiveness in normal infants and young children. Am J Respir Crit Care Med 2001; 164(3): 447-54.

628. Modl M, Eber E, Malle-Scheid D, Weinhandl E, Zach MS. Does bronchodilator responsiveness in infants with bronchiolitis depend on age? J Pediatr 2005; 147(5): 617-21.

629. Prendiville A, Rose A, Maxwell DL, Silverman M. Hypoxaemia in wheezy infants after bronchodilator treatment. Arch Dis Child 1987; 62(10): 997-1000.

630. Prendiville A, Green S, Silverman M. Paradoxical response to nebulised salbutamol in wheezy infants, assessed by partial expiratory flow-volume curves. Thorax 1987; 42(2): 86-91.

631. Hyvarinen MK, Kotaniemi-Syrjanen A, Reijonen TM, Korhonen K, Kiviniemi V, Korppi M. Responses to inhaled bronchodilators in infancy are not linked with asthma in later childhood. Pediatr Pulmonol 2006; 41(5): 420-7.

632. Debley J, Stanojevic S, Filbrun AG, Subbarao P. Bronchodilator responsiveness in wheezy infants and toddlers is not associated with asthma risk factors. Pediatr Pulmonol 2012; 47(5): 421-8.

633. Martinez FD, Graves PE, Baldini M, Solomon S, Erickson R. Association between genetic polymorphisms of the beta2-adrenoceptor and response to albuterol in children with and without a history of wheezing. J Clin Invest 1997; 100(12): 3184-8.

634. Rosenfeld M, Allen J, Arets BH, et al. An official American Thoracic Society workshop report: optimal lung function tests for monitoring cystic fibrosis, bronchopulmonary dysplasia, and recurrent wheezing in children less than 6 years of age. Ann Am Thorac Soc 2013; 10(2): S1-s11.

635. Glyn J. The discovery and early use of cortisone. J R Soc Med 1998; 91(10): 513-7.

636. Czock D, Keller F, Rasche FM, Haussler U. Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids. Clin Pharmacokinet 2005; 44(1): 61-98.

637. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. N Engl J Med 2005; 353(16): 1711-23.

638. Barnes PJ. Glucocorticosteroids: current and future directions. Br J Pharmacol 2011; 163(1): 29-43.

639. McDonough AK, Curtis JR, Saag KG. The epidemiology of glucocorticoid-associated adverse events. Curr Opin Rheumatol 2008; 20(2): 131-7.

640. Shinwell ES, Eventov-Friedman S. Impact of perinatal corticosteroids on neuromotor development and outcome: review of the literature and new meta-analysis. Semin Fetal Neonatal Med 2009; 14(3): 164-70.

641. Fernandes RM, Oleszczuk M, Woods CR, Rowe BH, Cates CJ, Hartling L. The Cochrane Library and safety of systemic corticosteroids for acute respiratory conditions in children: an overview of reviews. Evid Based Child Health 2014; 9(3): 733-47.

642. Hendeles L. Selecting a systemic corticosteroid for acute asthma in young children. J Pediatr 2003; 142(2 Suppl): S40-4.

643. Somers CC, Ahmad N, Mejias A, et al. Effect of dexamethasone on respiratory syncytial virus-induced lung inflammation in children: results of a randomized, placebo controlled clinical trial. Pediatr Allergy Immunol 2009; 20(5): 477-85.

644. Hinzey A, Alexander J, Corry J, et al. Respiratory syncytial virus represses glucocorticoid receptor-mediated gene activation. Endocrinology 2011; 152(2): 483-94.

645. Webster Marketon JI, Corry J, Teng MN. The respiratory syncytial virus (RSV) nonstructural proteins mediate RSV suppression of glucocorticoid receptor transactivation. Virology 2014; 449: 62-9.

646. Lehtinen P, Ruohola A, Vanto T, Vuorinen T, Ruuskanen O, Jartti T. Prednisolone reduces recurrent wheezing after a first wheezing episode associated with rhinovirus infection or eczema. J Allergy Clin Immunol 2007; 119(3): 570-5.

647. Bush A. Practice imperfect--treatment for wheezing in preschoolers. N Engl J Med. United States; 2009: 409-10.

648. Panickar J, Lakhanpaul M, Lambert PC, et al. Oral prednisolone for preschool children with acute virus-induced wheezing. N Engl J Med 2009; 360(4): 329-38.

649. Ducharme FM, Krajinovic M. Steroid responsiveness and wheezing phenotypes. Paediatr Respir Rev 2011; 12(3): 170-6.

650. Black JL, Oliver BG, Roth M. Molecular mechanisms of combination therapy with inhaled corticosteroids and long-acting beta-agonists. Chest 2009; 136(4): 1095-100.

651. Schmidt M, Michel MC. How Can 1 + 1 = 3? β2-Adrenergic and Glucocorticoid Receptor Agonist Synergism in Obstructive Airway Diseases. Mol Pharmacol 2011; 80(6): 955-8.

652. Egger M, Smith GD, O'Rourke K. Introduction: Rationale, Potentials, and Promise of Systematic Reviews. Systematic Reviews in Health Care: BMJ Publishing Group; 2001: 1-19.

653. McKenzie JE, Salanti G, Lewis SC, Altman DG. Meta-analysis and The Cochrane Collaboration: 20 years of the Cochrane Statistical Methods Group. Systematic reviews 2013; 2: 80.

654. Gadomski AM, Bhasale AL. Bronchodilators for bronchiolitis. Cochrane Database Syst Rev 2006; (3): Cd001266.

655. Patel H, Platt R, Lozano JM. WITHDRAWN: Glucocorticoids for acute viral bronchiolitis in infants and young children. Cochrane Database Syst Rev 2008; (1): Cd004878.

656. Chung M, Newberry SJ, Ansari MT, et al. Two methods provide similar signals for the need to update systematic reviews. J Clin Epidemiol 2012; 65(6): 660-8.

657. Gurusamy KS, Gluud C, Nikolova D, Davidson BR. Design of surgical randomized controlled trials involving multiple interventions. J Surg Res 2011; 165(1): 118-27.

658. McAlister FA, Straus SE, Sackett DL, Altman DG. Analysis and reporting of factorial trials: a systematic review. J Am Med Assoc 2003; 289(19): 2545-53.

659. Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 2008; 336(7650): 924-6.

660. Higgins JP, Altman DG, Gotzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ 2011; 343: d5928.

661. Ioannidis JP. Integration of evidence from multiple meta-analyses: a primer on umbrella reviews, treatment networks and multiple treatments meta-analyses. CMAJ 2009; 181(8): 488-93.

662. Hartling L, Vandermeer B, Fernandes RM. Systematic reviews, overviews of reviews and comparative effectiveness reviews: a discussion of approaches to knowledge synthesis. Evid Based Child Health 2014; 9(2): 486-94.

663. Thomson D, Foisy M, Oleszczuk M, Wingert A, Chisholm A, Hartling L. Overview of reviews in child health: evidence synthesis and the knowledge base for a specific population. Evid Based Child Health 2013; 8(1): 3-10.

664. Salanti G, Higgins JP, Ades AE, Ioannidis JP. Evaluation of networks of randomized trials. Stat Methods Med Res 2008; 17(3): 279-301.

665. Song F, Xiong T, Parekh-Bhurke S, et al. Inconsistency between direct and indirect comparisons of competing interventions: meta-epidemiological study. BMJ 2011; 343: d4909.

666. Cipriani A, Higgins JP, Geddes JR, Salanti G. Conceptual and technical challenges in network meta-analysis. Ann Intern Med 2013; 159(2): 130-7.

667. Donegan S, Williamson P, D'Alessandro U, Tudur Smith C. Assessing key assumptions of network meta-analysis: a review of methods. Research Synthesis Methods 2013; 4(4): 291-323.

668. Tugwell P, Boers M. OMERACT conference on outcome measures in rheumatoid arthritis clinical trials: introduction. J Rheumatol 1993; 20(3): 528-30.

669. Gabriel SE, Normand S-LT. Getting the Methods Right — The Foundation of Patient-Centered Outcomes Research. N Engl J Med 2012; 367(9): 787-90.

670. Streiner DL, Norman GR. Health measurement scales a practical guide to their development and use. Oxford: Oxford University Press; 2008.

671. De Vet HCW, Terwee CB, Mokkink LB, Knol DL. Measurement in Medicine a Practical Guide. Cambridge: Cambridge University Press; 2011.

672. Clarke M. Standardising outcomes for clinical trials and systematic reviews. Trials 2007; 8: 39.

673. Williamson P, Altman D, Blazeby J, Clarke M, Gargon E. Driving up the quality and relevance of research through the use of agreed core outcomes. J Health Serv Res Policy 2012; 17(1): 1-2.

674. Zhang B, Schmidt B. Do we measure the right end points? A systematic review of primary outcomes in recent neonatal randomized clinical trials. J Pediatr 2001; 138(1): 76-80.

675. Thornley B, Adams C. Content and quality of 2000 controlled trials in schizophrenia over 50 years. BMJ 1998; 317(7167): 1181-4.

676. Miyar J, Adams CE. Content and quality of 10,000 controlled trials in schizophrenia over 60 years. Schizophr Bull 2013; 39(1): 226-9.

677. Potter S, Brigic A, Whiting PF, et al. Reporting Clinical Outcomes of Breast Reconstruction: A Systematic Review. J Natl Cancer Inst 2011; 103(1): 31-46.

678. Chan AW, Hrobjartsson A, Haahr MT, Gotzsche PC, Altman DG. Empirical evidence for selective reporting of outcomes in randomized trials: comparison of protocols to published articles. J Am Med Assoc 2004; 291(20): 2457-65.

679. Dwan K, Altman DG, Arnaiz JA, et al. Systematic review of the empirical evidence of study publication bias and outcome reporting bias. PLoS One 2008; 3(8): e3081.

680. Kirkham JJ, Dwan KM, Altman DG, et al. The impact of outcome reporting bias in randomised controlled trials on a cohort of systematic reviews. BMJ 2010; 340: c365.

681. Tugwell P, Boers M, Brooks P, Simon L, Strand V, Idzerda L. OMERACT: an international initiative to improve outcome measurement in rheumatology. Trials 2007; 8: 38.

682. Williamson PR, Altman DG, Blazeby JM, et al. Developing core outcome sets for clinical trials: issues to consider. Trials 2012; 13: 132.

683. Guyatt GH, Oxman AD, Kunz R, et al. GRADE guidelines: 2. Framing the question and deciding on important outcomes. J Clin Epidemiol 2011; 64(4): 395-400.

684. Boers M, Brooks P, Strand CV, Tugwell P. The OMERACT filter for Outcome Measures in Rheumatology. J Rheumatol 1998; 25(2): 198-9.

685. Turk DC, Dworkin RH, Allen RR, et al. Core outcome domains for chronic pain clinical trials: IMMPACT recommendations. Pain 2003; 106(3): 337-45.

686. Mercuri E, Mayhew A, Muntoni F, et al. Towards harmonisation of outcome measures for DMD and SMA within TREAT-NMD; report of three expert workshops: TREAT-NMD/ENMC workshop on outcome measures, 12th--13th May 2007, Naarden, The Netherlands; TREAT-NMD workshop on outcome measures in experimental trials for DMD, 30th June--1st July 2007, Naarden, The Netherlands; conjoint Institute of Myology TREAT-NMD meeting on physical activity monitoring in neuromuscular disorders, 11th July 2007, Paris, France. Neuromuscul Disord. England; 2008: 894-903.

687. Schmitt J, Williams H. Harmonising Outcome Measures for Eczema (HOME). Report from the First International Consensus Meeting (HOME 1), 24 July 2010, Munich, Germany. Br J Dermatol 2010; 163(6): 1166-8.

688. Gargon E, Gurung B, Medley N, et al. Choosing important health outcomes for comparative effectiveness research: a systematic review. PLoS One 2014; 9(6): e99111.

689. Sinha I, Jones L, Smyth RL, Williamson PR. A systematic review of studies that aim to determine which outcomes to measure in clinical trials in children. PLoS Med 2008; 5(4): e96.

690. Gargon E, Williamson PR, Altman DG, Blazeby JM, Clarke M. The COMET Initiative database: progress and activities from 2011 to 2013. Trials 2014; 15(1): 279.

691. Boers M, Kirwan JR, Wells G, et al. Developing core outcome measurement sets for clinical trials: OMERACT filter 2.0. J Clin Epidemiol 2014; 67(7): 745-53.

692. Idzerda L, Rader T, Tugwell P, Boers M. Can We Decide Which Outcomes Should Be Measured in Every Clinical Trial? A Scoping Review of the Existing Conceptual Frameworks and Processes to Develop Core Outcome Sets. J Rheumatol 2014; 41(5): 986-93.

693. Klassen TP. Determining the benefit of bronchodilators in bronchiolitis. When is there enough benefit to warrant adoption into clinical practice? Arch Pediatr Adolesc Med 1996; 150(11): 1120-1.

694. Bekhof J, Reimink R, Brand PL. Systematic review: insufficient validation of clinical scores for the assessment of acute dyspnoea in wheezing children. Paediatr Respir Rev 2014; 15(1): 98-112.

695. Viswanathan M, King VJ, Bordley C, et al. Management of bronchiolitis in infants and children. Evidence report/technology assessment (Summary) 2003; (69): 1-5.

696. Bakas T, McLennon SM, Carpenter JS, et al. Systematic review of health-related quality of life models. Health Qual Life Outcomes 2012; 10: 134.

697. Wilson IB, Cleary PD. Linking clinical variables with health-related quality of life. A conceptual model of patient outcomes. J Am Med Assoc 1995; 273(1): 59-65.

698. Ferrans CE, Zerwic JJ, Wilbur JE, Larson JL. Conceptual model of health-related quality of life. J Nurs Scholarsh 2005; 37(4): 336-42.

699. Sinha IP, Altman DG, Beresford MW, et al. Standard 5: Selection, Measurement, and Reporting of Outcomes in Clinical Trials in Children. Pediatrics 2012; 129(Supplement 3): S146-S52.

700. Zarin DA, Tse T, Williams RJ, Califf RM, Ide NC. The ClinicalTrials.gov results database--update and key issues. N Engl J Med 2011; 364(9): 852-60.

701. Mokkink LB, Terwee CB, Patrick DL, et al. The COSMIN study reached international consensus on taxonomy, terminology, and definitions of measurement properties for health-related patient-reported outcomes. J Clin Epidemiol 2010; 63(7): 737-45.

702. Streiner DL. Clinimetrics vs. psychometrics: an unnecessary distinction. J Clin Epidemiol 2003; 56(12): 1142-5; discussion 6-9.

703. de Vet HC, Terwee CB, Bouter LM. Current challenges in clinimetrics. J Clin Epidemiol 2003; 56(12): 1137-41.

704. Mokkink LB, Terwee CB, Patrick DL, et al. The COSMIN checklist for assessing the methodological quality of studies on measurement properties of health status measurement instruments: an international Delphi study. Qual Life Res 2010; 19(4): 539-49.

705. Terwee CB, Mokkink LB, Knol DL, Ostelo RW, Bouter LM, de Vet HC. Rating the methodological quality in systematic reviews of studies on measurement properties: a scoring system for the COSMIN checklist. Qual Life Res 2012; 21(4): 651-7.

706. Terwee CB, Dekker FW, Wiersinga WM, Prummel MF, Bossuyt PM. On assessing responsiveness of health-related quality of life instruments: guidelines for instrument evaluation. Qual Life Res 2003; 12(4): 349-62.

707. Jaeschke R, Singer J, Guyatt GH. Measurement of health status. Ascertaining the minimal clinically important difference. Control Clin Trials 1989; 10(4): 407-15.

708. de Vet HC, Terwee CB, Ostelo RW, Beckerman H, Knol DL, Bouter LM. Minimal changes in health status questionnaires: distinction between minimally detectable change and minimally important change. Health Qual Life Outcomes 2006; 4: 54.

709. Terwee CB, Roorda LD, Knol DL, De Boer MR, De Vet HC. Linking measurement error to minimal important change of patient-reported outcomes. J Clin Epidemiol 2009; 62(10): 1062-7.

710. Terwee CB, Roorda LD, Dekker J, et al. Mind the MIC: large variation among populations and methods. J Clin Epidemiol 2010; 63(5): 524-34.

711. Turner D, Schunemann HJ, Griffith LE, et al. The minimal detectable change cannot reliably replace the minimal important difference. J Clin Epidemiol 2010; 63(1): 28-36.

712. Juniper EF, Guyatt GH, Willan A, Griffith LE. Determining a minimal important change in a disease-specific Quality of Life Questionnaire. J Clin Epidemiol 1994; 47(1): 81-7.

713. Schunemann HJ, Guyatt GH. Commentary--goodbye M(C)ID! Hello MID, where do you come from? Health Serv Res 2005; 40(2): 593-7.

714. Lowell DI, Lister G, Von Koss H, McCarthy P. Wheezing in infants: the response to epinephrine. Pediatrics 1987; 79(6): 939-45.

715. van der Windt DA, Nagelkerke AF, Bouter LM, Dankert-Roelse JE, Veerman AJ. Clinical scores for acute asthma in pre-school children. A review of the literature. J Clin Epidemiol 1994; 47(6): 635-46.

716. Birken CS, Parkin PC, Macarthur C. Asthma severity scores for preschoolers displayed weaknesses in reliability, validity, and responsiveness. J Clin Epidemiol 2004; 57(11): 1177-81.

717. Destino L, Weisgerber MC, Soung P, et al. Validity of respiratory scores in bronchiolitis. Hosp Pediatr 2012; 2(4): 202-9.

718. Horowitz L. Bronchiolitis and Asthma. Pediatrics 1967; 40(4): 693-4.

719. Court SDM. The definition of acute respiratory illnesses in children. Postgrad Med J 1973; 49(577): 771-6.

720. McIntosh K. Bronchiolitis and asthma: Possible common pathogenetic pathways. J Allergy Clin Immunol 1976; 57(6): 595-604.

721. McConnochie KM. Bronchiolitis. What's in the name? Am J Dis Child (1960) 1983; 137(1): 11-3.

722. Milner AD, Murray M. Acute bronchiolitis in infancy: treatment and prognosis. Thorax 1989; 44(1): 1-5.

723. Wilson NM. Wheezy bronchitis revisited. Arch Dis Child 1989; 64(8): 1194-9.

724. Ruuskanen O, Ogra PL. Respiratory syncytial virus. Curr Probl Pediatr 1993; 23(2): 50-79.

725. Korppi M, Koponen P, Nuolivirta K. Upper age limit for bronchiolitis: 12 months or 6 months? Eur Respir J 2012; 39(3): 787-8.

726. Lakhanpaul M, Armon K, Eccleston P, et al. An evidence based guideline for the management of children presenting with acute breathing difficulty University of Nottingham: Nottingham. http://www.nottingham.ac.uk/paediatric-guideline/breathingguideline.pdf.

727. Hall CB. Dexamethasone of no benefit in moderate-to-severe bronchiolitis. J Pediatr 2008; 152(1): 143-4.

728. Guilbert TW, Bacharier LB. Controversies in the treatment of the acutely wheezing infant. Am J Respir Crit Care Med. United States; 2011: 1284-5.

729. Aalseth PT. Medical coding : what it is and how it works. Sudbury, Mass.: Jones and Bartlett Publishers; 2006.

730. Ryu JH, Myers JL, Swensen SJ. Bronchiolar disorders. Am J Respir Crit Care Med 2003; 168(11): 1277-92.

731. Elphick HE, Sherlock P, Foxall G, et al. Survey of respiratory sounds in infants. Arch Dis Child 2001; 84(1): 35-9.

732. Cane RS, Ranganathan SC, McKenzie SA. What do parents of wheezy children understand by “wheeze”? Arch Dis Child 2000; 82(4): 327-32.

733. Fernandes R, Robalo B, Calado C, et al. The multiple meanings of "wheezing": a questionnaire survey in Portuguese for parents and health professionals. BMC Pediatr 2011; 11(1): 1-9.

734. Elphick HE, Ritson S, Rigby AS, Everard ML. Phenotype of acute respiratory syncytial virus induced lower respiratory tract illness in infancy and subsequent morbidity. Acta Paediatr 2007; 96(2): 307-9.

735. Fouzas S, Brand PLP. Predicting persistence of asthma in preschool wheezers: crystal balls or muddy waters? Paediatr Respir Rev 2013; 14(1): 46-52.

736. Wardlaw AJ, Silverman M, Siva R, Pavord ID, Green R. Multi-dimensional phenotyping: towards a new taxonomy for airway disease. Clin Exp Allergy 2005; 35(10): 1254-62.

737. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet 2008; 372(9643): 1107-19.

738. Agache I, Akdis C, Jutel M, Virchow JC. Untangling asthma phenotypes and endotypes. Allergy 2012; 67(7): 835-46.

739. Haldar P, Pavord ID, Shaw DE, et al. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med 2008; 178(3): 218-24.

740. Spycher BD, Silverman M, Brooke AM, Minder CE, Kuehni CE. Distinguishing phenotypes of childhood wheeze and cough using latent class analysis. Eur Respir J 2008; 31(5): 974-81.

741. Weatherall M, Shirtcliffe P, Travers J, Beasley R. Use of cluster analysis to define COPD phenotypes. Eur Respir J 2010; 36(3): 472-4.

742. Spycher BD, Silverman M, Barben J, et al. A Disease Model for Wheezing Disorders in Preschool Children Based on Clinicians' Perceptions. PLoS ONE 2009; 4(12): e8533.

743. Turner T, Wilkinson F, Harris C, Mazza D, Health for Kids Guideline Development G. Evidence based guideline for the management of bronchiolitis. Aust Fam Physician 2008; 37(6 Spec No): 6-13.

744. Muething S, Schoettker PJ, Gerhardt WE, Atherton HD, Britto MT, Kotagal UR. Decreasing overuse of therapies in the treatment of bronchiolitis by incorporating evidence at the point of care. J Pediatr 2004; 144(6): 703-10.

745. Touzet S, Refabert L, Letrilliart L, Ortolan B, Colin C. Impact of consensus development conference guidelines on primary care of bronchiolitis: are national guidelines being followed? J Eval Clin Pract 2007; 13(4): 651-6.

746. Shojania KG, Sampson M, Ansari MT, Ji J, Doucette S, Moher D. How quickly do systematic reviews go out of date? A survival analysis. Ann Intern Med 2007; 147(4): 224-33.

747. Schuh S, Coates AL, Binnie R, et al. Efficacy of oral dexamethasone in outpatients with acute bronchiolitis. J Pediatr 2002; 140(1): 27-32.

748. Caldwell DM, Ades AE, Higgins JP. Simultaneous comparison of multiple treatments: combining direct and indirect evidence. BMJ 2005; 331(7521): 897-900.

749. Cochrane Handbook for Systematic Reviews of Interventions. In: Higgins JPT GS, editor. 2011 ed: The Cochrane Collaboration.

750. Owens DK, Lohr KN, Atkins D, et al. AHRQ series paper 5: grading the strength of a body of evidence when comparing medical interventions--agency for healthcare research and quality and the effective health-care program. J Clin Epidemiol 2010; 63(5): 513-23.

751. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7(3): 177-88.

752. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002; 21(11): 1539-58.

753. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003; 327(7414): 557-60.

754. Higgins JP, Whitehead A. Borrowing strength from external trials in a meta-analysis. Stat Med 1996; 15(24): 2733-49.

755. Lu G, Ades AE. Combination of direct and indirect evidence in mixed treatment comparisons. Stat Med 2004; 23(20): 3105-24.

756. Smith TC, Spiegelhalter DJ, Thomas A. Bayesian approaches to random-effects meta-analysis: a comparative study. Stat Med 1995; 14(24): 2685-99.

757. Dias S, Welton NJ, Caldwell DM, Ades AE. Checking consistency in mixed treatment comparison meta-analysis. Stat Med 2010; 29(7-8): 932-44.

758. Glasziou PP, Shepperd S, Brassey J. Can we rely on the best trial? A comparison of individual trials and systematic reviews. BMC Med Res Methodol 2010; 10: 23.

759. Hartling L, Wiebe N, Russell K, Patel H, Klassen TP. Epinephrine for bronchiolitis. Cochrane Database Syst Rev 2004; (1): CD003123.

760. Wohl ME, Chernick V. State of the art: bronchiolitis. Am Rev Respir Dis 1978; 118(4): 759-81.

761. Thomas LH, Sharland M, Friedland JS. Steroids fail to down-regulate respiratory syncytial virus-induced IL-8 secretion in infants. Pediatric research 2002; 52(3): 368-72.

762. Openshaw PJ. Antiviral immune responses and lung inflammation after respiratory syncytial virus infection. Proc Am Thorac Soc 2005; 2(2): 121-5.

763. Giembycz MA, Kaur M, Leigh R, Newton R. A Holy Grail of asthma management: toward understanding how long-acting beta(2)-adrenoceptor agonists enhance the clinical efficacy of inhaled corticosteroids. Br J Pharmacol 2008; 153(6): 1090-104.

764. Frey U, von Mutius E. The challenge of managing wheezing in infants. N Engl J Med. United States; 2009: 2130-3.

765. Karemaker R, Kavelaars A, ter Wolbeek M, et al. Neonatal dexamethasone treatment for chronic lung disease of prematurity alters the hypothalamus-pituitary-adrenal axis and immune system activity at school age. Pediatrics 2008; 121(4): e870-8.

766. Karemaker R, Karemaker JM, Kavelaars A, et al. Effects of neonatal dexamethasone treatment on the cardiovascular stress response of children at school age. Pediatrics 2008; 122(5): 978-87.

767. Onland W, De Jaegere AP, Offringa M, van Kaam AH. Effects of higher versus lower dexamethasone doses on pulmonary and neurodevelopmental sequelae in preterm infants at risk for chronic lung disease: a meta-analysis. Pediatrics 2008; 122(1): 92-101.

768. Wilson-Costello D, Walsh MC, Langer JC, et al. Impact of postnatal corticosteroid use on neurodevelopment at 18 to 22 months' adjusted age: effects of dose, timing, and risk of bronchopulmonary dysplasia in extremely low birth weight infants. Pediatrics 2009; 123(3): e430-7.

769. Halliday HL, Ehrenkranz RA, Doyle LW. Early (< 8 days) postnatal corticosteroids for preventing chronic lung disease in preterm infants. Cochrane Database Syst Rev 2010; (1): CD001146.

770. Flores G, Horwitz RI. Efficacy of beta2-agonists in bronchiolitis: a reappraisal and meta-analysis. Pediatrics 1997; 100(2 Pt 1): 233-9.

771. Barbi E, Neri E, Ventura A. Nomina sunt consequentia rerum: time for a change in the definition of bronchiolitis? Arch Pediatr Adolesc Med 2004; 158(4): 403; author reply

772. Yusuf S, Piedimonte G, Auais A, et al. The relationship of meteorological conditions to the epidemic activity of respiratory syncytial virus. Epidemiol Infect 2007; 135(7): 1077-90.

773. Meissner HC. Selected populations at increased risk from respiratory syncytial virus infection. Pediatr Infect Dis J 2003; 22(2 Suppl): S40-4; discussion S4-5.

774. Singh AM, Moore PE, Gern JE, Lemanske RF, Jr., Hartert TV. Bronchiolitis to asthma: a review and call for studies of gene-virus interactions in asthma causation. Am J Respir Crit Care Med 2007; 175(2): 108-19.

775. Hartling L, Fernandes RM, Bialy L, et al. Steroids and bronchodilators for acute bronchiolitis in the first two years of life: systematic review and meta-analysis. BMJ 2011; 342: d1714.

776. Zhang L, Mendoza-Sassi RA, Wainwright C, Klassen TP. Nebulised hypertonic saline solution for acute bronchiolitis in infants. Cochrane Database Syst Rev 2013; 7: Cd006458.

777. Hall CB. Therapy for bronchiolitis: when some become none. N Engl J Med 2007; 357(4): 402-4.

778. Halfhide C, Smyth RL. Innate immune response and bronchiolitis and preschool recurrent wheeze. Paediatr Respir Rev 2008; 9(4): 251-62.

779. Buckingham SC, Jafri HS, Bush AJ, et al. A randomized, double-blind, placebo-controlled trial of dexamethasone in severe respiratory syncytial virus (RSV) infection: effects on RSV quantity and clinical outcome. J Infect Dis 2002; 185(9): 1222-8.

780. Barben JU, Robertson CF, Robinson PJ. Implementation of evidence-based management of acute bronchiolitis. J Paediatr Child Health 2000; 36(5): 491-7.

781. Montgomery AA, Peters TJ, Little P. Design, analysis and presentation of factorial randomised controlled trials. BMC Med Res Methodol 2003; 3: 26.

782. Follmann D, Elliott P, Suh I, Cutler J. Variance imputation for overviews of clinical trials with continuous response. J Clin Epidemiol 1992; 45(7): 769-73.

783. Wiebe N, Vandermeer B, Platt RW, Klassen TP, Moher D, Barrowman NJ. A systematic review identifies a lack of standardization in methods for handling missing variance data. J Clin Epidemiol 2006; 59(4): 342-53.

784. Weinberger M. Corticosteroids for first-time young wheezers: current status of the controversy. J Pediatr 2003; 143(6): 700-2.

785. Sly PD, Boner AL, Bjorksten B, et al. Early identification of atopy in the prediction of persistent asthma in children. Lancet 2008; 372(9643): 1100-6.

786. Schultz A, Devadason SG, Savenije OE, Sly PD, Le Souef PN, Brand PL. The transient value of classifying preschool wheeze into episodic viral wheeze and multiple trigger wheeze. Acta Paediatr 2010; 99(1): 56-60.

787. Castro-Rodriguez JA, Holberg CJ, Wright AL, Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. Am J Respir Crit Care Med 2000; 162(4 Pt 1): 1403-6.

788. Stein RT, Sherrill D, Morgan WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet 1999; 354(9178): 541-5.

789. Korppi M. Are responses to treatment virus-specific in wheezing children? J Allergy Clin Immunol 2007; 119(6): 1561-2.

790. Simpson A, Tan VY, Winn J, et al. Beyond atopy: multiple patterns of sensitization in relation to asthma in a birth cohort study. Am J Respir Crit Care Med 2010; 181(11): 1200-6.

791. Bjornson CL, Johnson DW. Croup. Lancet 2008; 371(9609): 329-39.

792. Schuh S, Coates AL, Dick P, et al. A single versus multiple doses of dexamethasone in infants wheezing for the first time. Pediatr Pulmonol 2008; 43(9): 844-50.

793. Hartling L, Bialy LM, Vandermeer B, et al. Epinephrine for bronchiolitis. Cochrane Database Syst Rev 2011; (6): CD003123.

794. Sinha IP, Williamson PR, Smyth RL. Outcomes in clinical trials of inhaled corticosteroids for children with asthma are narrowly focussed on short term disease activity. PLoS One 2009; 4(7): e6276.

795. Zorc JJ, Hall CB. Bronchiolitis: Recent Evidence on Diagnosis and Management. Pediatrics 2010; 125(2): 342-9.

796. de Vet HC, Terwee CB, Knol DL, Bouter LM. When to use agreement versus reliability measures. J Clin Epidemiol 2006; 59(10): 1033-9.

797. Klassen TP, Sutcliffe T, Watters LK, Wells GA, Allen UD, Li MM. Dexamethasone in salbutamol-treated inpatients with acute bronchiolitis: a randomized, controlled trial. J Pediatr 1997; 130(2): 191-6.

798. Zhang L, Mendoza-Sassi R, Santos JC, Lau J. Accuracy of symptoms and signs in predicting hypoxaemia among young children with acute respiratory infection: a meta-analysis. Int J Tuberc Lung Dis 2011; 15(3): 317-25.

799. Chalut DS, Ducharme FM, Davis GM. The Preschool Respiratory Assessment Measure (PRAM): a responsive index of acute asthma severity. J Pediatr 2000; 137(6): 762-8.

800. Gorelick MH, Stevens MW, Schultz TR, Scribano PV. Performance of a novel clinical score, the Pediatric Asthma Severity Score (PASS), in the evaluation of acute asthma. Acad Emerg Med 2004; 11(1): 10-8.

801. Jolliffe IT. Principal Components Used with Other Multivariate Techniques. Principal Component Analysis: Springer New York; 2002: 199-231.

802. Midulla F, Nenna R. Upper age limit for bronchiolitis: 12 months or 6 months? Eur Respir J 2012; 39(3): 788-9.

803. Walsh P, Rothenberg SJ, Robbins D, Caldwell J, Friese S, Garzon A. Flawed meta-analysis creates doubt when answers are known. BMJ 2011; 342: d3348.

804. Riley RD, Lambert PC, Abo-Zaid G. Meta-analysis of individual participant data: rationale, conduct, and reporting. BMJ 2010; 340: c221.

805. Mills EJ, Thorlund K, Ioannidis JP. Calculating additive treatment effects from multiple randomized trials provides useful estimates of combination therapies. J Clin Epidemiol 2012; 65(12): 1282-8.

806. Nikolakopoulou A, Chaimani A, Veroniki AA, Vasiliadis HS, Schmid CH, Salanti G. Characteristics of networks of interventions: a description of a database of 186 published networks. PLoS One 2014; 9(1): e86754.

807. Salanti G, Ades AE, Ioannidis JP. Graphical methods and numerical summaries for presenting results from multiple-treatment meta-analysis: an overview and tutorial. J Clin Epidemiol 2011; 64(2): 163-71.

808. Jansen JP, Schmid CH, Salanti G. Directed acyclic graphs can help understand bias in indirect and mixed treatment comparisons. J Clin Epidemiol 2012; 65(7): 798-807.

809. Salanti G, Del Giovane C, Chaimani A, Caldwell DM, Higgins JP. Evaluating the quality of evidence from a network meta-analysis. PLoS One 2014; 9(7): e99682.

810. Elliott JH, Turner T, Clavisi O, et al. Living systematic reviews: an emerging opportunity to narrow the evidence-practice gap. PLoS Med 2014; 11(2): e1001603.

811. Thorlund K, Anema A, Mills E. Interpreting meta-analysis according to the adequacy of sample size. An example using isoniazid chemoprophylaxis for

tuberculosis in purified protein derivative negative HIV-infected individuals. Clinical epidemiology 2010; 2: 57-66.

812. Mandelberg A, Amirav I. Hypertonic saline or high volume normal saline for viral bronchiolitis: mechanisms and rationale. Pediatr Pulmonol 2010; 45(1): 36-40.

813. Skjerven HO, Hunderi JOG, Brügmann-Pieper SK, et al. Racemic Adrenaline and Inhalation Strategies in Acute Bronchiolitis. N Engl J Med 2013; 368(24): 2286-93.

814. Haq I, Gopalakaje S, Fenton AC, McKean MC, C JOB, Brodlie M. The evidence for high flow nasal cannula devices in infants. Paediatr Respir Rev 2014; 15(2): 124-34.

815. Kirkham JJ, Boers M, Tugwell P, Clarke M, Williamson PR. Outcome measures in rheumatoid arthritis randomised trials over the last 50 years. Trials 2013; 14: 324.

816. Prinsen CA, Vohra S, Rose MR, et al. Core Outcome Measures in Effectiveness Trials (COMET) initiative: protocol for an international Delphi study to achieve consensus on how to select outcome measurement instruments for outcomes included in a 'core outcome set'. Trials 2014; 15: 247.

817. van Miert C, Abbott J, Verheoff F, Lane S, Carter B, McNamara P. Development and validation of the Liverpool infant bronchiolitis severity score: a research protocol. J Adv Nurs 2014; 70(10): 2353-62.

818. Brozek JL, Guyatt GH, Schunemann HJ. How a well-grounded minimal important difference can enhance transparency of labelling claims and improve interpretation of a patient reported outcome measure. Health Qual Life Outcomes. England; 2006: 69.

819. Johnston BC, Thorlund K, da Costa BR, Furukawa TA, Guyatt GH. New methods can extend the use of minimal important difference units in meta-analyses of continuous outcome measures. J Clin Epidemiol 2012; 65(8): 817-26.

820. Silverman M, Wilson N. Asthma—time for a change of name? Arch Dis Child 1997; 77(1): 62-5.

821. Rome F. Guidelines--Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders. J Gastrointestin Liver Dis 2006; 15(3): 307-12.

APPENDIX

A1 Supplementary Data For Network Meta-Analysis

A2 Supplementary Data For Cochrane Review

A3 Formulas

A4 Questionnaires (Pediatricians And GPs), Ethics Comittee Approval

A1 SUPPLEMENTARY DATA FOR NETWORK META-ANALYSIS (CHAPTER 2.1)

LIST OF INCLUDED STUDIES

Abu-Shukair M. Salbutamol versus epinephrine in the treatment of acute bronchiolitis. Journal of the Bahrain Medical Society 2001;13(3):143-6.

Abul-Ainine A, Luyt D. Short term effects of adrenaline in bronchiolitis: a randomised controlled trial. Arch Dis Child 2002;86(4):276-9.

Barlas C, Kiper N, Göçmen A, Ozcelik U, Dilber E, Anadol D, et al. [Racemic adrenaline and other treatment regimens in mild and moderate bronchiolitis]. Cocuk Sagligi Hast Derg 1998;41(2):155-65. Turkish.

Beck R, Elias N, Shoval S, Tov N, Talmon G, Godfrey S, Bentur L. Computerized acoustic assessment of treatment efficacy of nebulized epinephrine and albuterol in RSV bronchiolitis. BMC Pediatr 2007;7(22).

Bentur L, Shoseyov D, Feigenbaum D, Gorichovsky Y, Bibi H. Dexamethasone inhalations in RSV bronchiolitis: a double-blind, placebo-controlled study. Acta Paediatr 2005;94(7):866-71.

Berger I, Argaman Z, Schwartz SB, Segal E, Kiderman A, Branski D, Kerem E. Efficacy of corticosteroids in acute bronchiolitis: short-term and long-term followup. Pediatr Pulmonol 1998;26(3):162-6.

Bertrand P, Aranibar H, Castro E, Sanchez I. Efficacy of nebulized epinephrine versus salbutamol in hospitalized infants with bronchiolitis. Pediatr Pulmonol 2001;31(4):284-8.

Bilan N, Saiied Sadry N. Clinical efficacy of salbutamol spray in treatment of bronchiolitis. Medical Journal of Tabriz University of Medical Sciences 2007;29(1):27-30.

Cade A, Brownlee KG, Conway SP, Haigh D, Short A, Brown J, et al. Randomised placebo controlled trial of nebulised corticosteroids in acute respiratory syncytial viral bronchiolitis. Arch Dis Child 2000;82(2):126-30.

Can D, Inan G, Yendur G, Oral R, Gunay I. Salbutamol or mist in acute bronchiolitis. Acta Paediatr Jpn 1998;40(3):252-5.

Cengizlier R, Saraclar Y, Adalioglu G, Tuncer A. Effect of oral and inhaled salbutamol in infants with bronchiolitis. Acta Paediatr Jpn 1997;39(1):61-3.

Chevallier B, Aegerter P, Parat S, Bidat E, Renaud C, Lagardère B. Comparative study of nebulized sambutol against placebo in the acute phase of bronchiolitis in 33 infants aged 1 to 6 months. Arch Pediatr 1995;2(1):11-7.

Chowdhury D, al Howasi M, Khalil M, al-Frayh AS, Chowdhury S, Ramia S. The role of bronchodilators in the management of bronchiolitis: a clinical trial. Ann Trop Paediatr 1995;15(1):77-84.

Corneli HM, Zorc JJ, Mahajan P, Shaw KN, Holubkov R, Reeves SD, et al. A multicenter, randomized, controlled trial of dexamethasone for bronchiolitis. N Engl J Med 2007;357(4):331-9.

De Boeck K, Van der Aa N, Van Lierde S, Corbeel L, Eeckels R. Respiratory syncytial virus bronchiolitis: a double-blind dexamethasone efficacy study. J Pediatr 1997;131(6):919-21.

Gadomski AM, Aref GH, el Din OB, el Sawy IH, Khallaf N, Black RE. Oral versus nebulized albuterol in the management of bronchiolitis in Egypt. J Pediatr 1994;124(1):131-8.

Gadomski AM, Lichenstein R, Horton L, King J, Keane V, Permutt T. Efficacy of albuterol in the management of bronchiolitis. Pediatrics 1994;93(6 Pt 1):907-12.

Goebel J, Estrada B, Quinonez J, Nagji N, Sanford D, Boerth RC. Prednisolone plus

albuterol versus albuterol alone in mild to moderate bronchiolitis. Clin Pediatr (Phila) 2000;39(4):213-20.
Goh A, Chay OM, Foo AL, Ong EK. Efficacy of bronchodilators in the treatment of bronchiolitis. Singapore Med J 1997;38(8):326-8.

Gómez-y-López RE, Hernández-Sierra JF, Torres-Ruvalcaba BA, Martinez-Puente E, del Cármen Martinez-Garcia M. [Comparative clinical study of dexamethasone vs. nebulized salbutamol in acute bronchiolitis]. Gac Med Mex 2007;143(3): 189-92. Spanish.

Gupta P, Aggarwal A, Gupta P, Sharma KK. Oral salbutamol for symptomatic relief in mild bronchiolitis a double blind randomized placebo controlled trial. Indian Pediatr 2008;45(7):547-53.

Ho L, Collis G, Landau LI, Le Souef PN. Effect of salbutamol on oxygen saturation in bronchiolitis. Arch Dis Child 1991;66(9):1061-4.

John BM, Patnaik SK, Prasad PL. Efficacy of nebulised epinephrine versus salbutamol in hospitalised children with bronchiolitis. Medical Journal Armed Forces India 2006;62(4):354-7.

Karadag B, Ceran O, Guven G, Dursun E, Ipek IO, Karakoc F, et al. Efficacy of salbutamol and ipratropium bromide in the management of acute bronchiolitis-A clinical trial. Respiration 2008;76(3):283-7.

Khashabi J. Comparison of the efficacy of nebulized L-epinephrine, salbutamol and normal saline in acute bronchiolitis: a randomized clinical trial. Medical Journal of the Islamic Republic of Iran 2005;19(2):119-25.

Klassen TP, Rowe PC, Sutcliffe T, Ropp LJ, McDowell IW, Li MM. Randomized trial of salbutamol in acute bronchiolitis. J Pediatr 1991;118(5):807-11.

Klassen TP, Sutcliffe T, Watters LK, Wells GA, Allen UD, Li MM. Dexamethasone in salbutamol-treated inpatients with acute bronchiolitis: a randomized, controlled trial. J Pediatr 1997;130(2):191-6.

Kuyucu S, Unal S, Kuyucu N, Yilgor E. Additive effects of dexamethasone in nebulized salbutamol or L-epinephrine treated infants with acute bronchiolitis. Pediatr Int 2004;46(5):539-44.

Menon K, Sutcliffe T, Klassen TP. A randomized trial comparing the efficacy of epinephrine with salbutamol in the treatment of acute bronchiolitis. J Pediatr 1995;126(6):1004-7.

Mesquita M, Castro-Rodríguez JA, Heinichen L, Farina E, Iramain R. Single oral dose of dexamethasone in outpatients with bronchiolitis: a placebo controlled trial. Allergol Immunopathol 2009;37(2):63-7.

Mull CC, Scarfone RJ, Ferri LR, Carlin T, Salvaggio C, Bechtel KA, et al. A randomized trial of nebulized epinephrine vs albuterol in the emergency department treatment of bronchiolitis. Arch Pediatr Adolesc Med 2004;158(2): 113-8.

Okutan V, Akin R, Kurekci AE, Yanik A, Ozcan O, Gokcay E. [Effectiveness of nebulised adrenaline and salbutamol in the treatment of infants with bronchiolitis]. Bulletin of Gulhane Military Medical Academy 1998;40(2):199-204. Turkish.

Patel H, Gouin S, Platt RW. Randomized, double-blind, placebo-controlled trial of oral albuterol in infants with mild-to-moderate acute viral bronchiolitis. J Pediatr 2003;142(5):509-14.

Patel H, Platt RW, Pekeles GS, Ducharme FM. A randomized, controlled trial of the effectiveness of nebulized therapy with epinephrine compared with albuterol and saline in infants hospitalized for acute viral bronchiolitis. J Pediatr 2002;141(6):818-24.

Plint AC, Johnson DW, Patel H, Wiebe N, Correll R, Brant R, et al. Epinephrine and dexamethasone in children with bronchiolitis. N Engl J Med 2009;360(20):2079-89.

Ralston S, Hartenberger C, Anaya T, Qualls C, Kelly HW. Randomized, placebo-controlled trial of albuterol and epinephrine at equipotent beta-2 agonist doses in acute bronchiolitis. Pediatr Pulmonol 2005;40(4):292-9.

Richter H, Seddon P. Early nebulized budesonide in the treatment of bronchiolitis and the prevention of postbronchiolitic wheezing. J Pediatr 1998;132(5):849-53.

Roosevelt G, Sheehan K, Grupp-Phelan J, Tanz RR, Listernick R. Dexamethasone in bronchiolitis: a randomised controlled trial. Lancet 1996;348(9023):292-5.

Sanchez I, De Koster J, Powell RE, Wolstein R, Chernick V. Effect of racemic epinephrine and salbutamol on clinical score and pulmonary mechanics in infants with bronchiolitis. J Pediatr 1993;122(1):145-51.

Schuh S, Canny G, Reisman JJ, Kerem E, Bentur L, Petric M, Levison H. Nebulized albuterol in acute bronchiolitis. J Pediatr 1990;117(4):633-7.

Schuh S, Coates AL, Binnie R, Allin T, Goia C, Corey M, Dick PT. Efficacy of oral dexamethasone in outpatients with acute bronchiolitis. J Pediatr 2002;140(1):27-32.

Schuh S, Johnson D, Canny G, Reisman J, Shields M, Kovesi T, et al. Efficacy of adding nebulized ipratropium bromide to nebulized albuterol therapy in acute bronchiolitis. Pediatrics 1992;90(6):920-3.

Teeratakulpisarn J, Limwattananon C, Tanupattarachai S, Limwattananon S, Teeratakulpisarn S, Kosalaraksa P. Efficacy of dexamethasone injection for acute bronchiolitis in hospitalized children: a randomized, double-blind, placebo-controlled trial. Pediatr Pulmonol 2007;42(5):433-9.

Tinsa F, Ben Rhouma A., Ghaffari H, Boussetta K, Zouari B, Brini I, et al. A randomized, controlled trial of nebulized terbutaline for the first acute bronchiolitis in infants less than 12-months-old. Tunis Med 2009;87(3):200-3.

Totapally BR, Demerci C, Zureikat G, Nolan B. Tidal breathing flow-volume loops in bronchiolitis in infancy: the effect of albuterol [ISRCTN47364493]. Crit Care

2002;6(2):160-5.

Uyan AP, Ozyurek H, Keskin M, Afsar Y, Yilmaz E. Comparison of two different bronchodilators in the treatment of acute bronchiolitis. Cocuk Sagligi Hast Derg 2002;45(4):298-303.

Wainwright C, Altamirano L, Cheney M, Cheney J, Barber S, Price D, et al. A multicenter, randomized, double-blind, controlled trial of nebulized epinephrine in infants with acute bronchiolitis. N Engl J Med 2003;349(1):27-35.

Zhang L, Ferruzzi E, Bonfanti T, Auler MI, D'avila NE, Faria CS, et al. Long and short-term effect of prednisolone in hospitalized infants with acute bronchiolitis. J Paediatr Child Health 2003;39(7):548-51.

SEARCH STRATEGIES

PubMed® - U.S. National Library of Medicine

1950 to 2009 Searched: 09Mar09	Results: n/a
Searched for a couple of pre 1965 older articles identified through reference lists	

Scopus® - Elsevier B.V.

1966 to November 2009	Searched: 25Nov09 Results: 45
(((TITLE(bronchiolitis OR wheez*) AND TITLE-ABS-KEY(steroid* OR glucocorticoid* OR corticosteroid*))) AND KEY("epinephrine" OR "adrenaline" OR "albuterol" OR "corticosteroids" OR "hydrocortisone" OR "steroids" OR ("inhaled steroids") OR "salbutamol" OR "betamethasone" OR "beclomethasone" OR "dexamethasone" OR "steroid" OR ("inhaled budesonide") OR "glucocorticoids" OR "bronchodilator" OR ("steroid use") OR "prednisolone" OR "methylprednisone" OR ("oral prednisolone") OR "prednisone" OR "ipratropium" OR "terbutaline" OR "orciprenaline" OR "fenoterol" OR "aminophylline" OR "androstadienes" OR "hydrocortisone")) AND (TITLE-ABS-KEY("Clinical Trial" OR "Clinical Trials" OR "Randomized Controlled Trial*" OR "Random Allocation" OR "double-blind method" OR "single-blind method" OR placebos OR research design OR comparative study OR evaluation studies OR follow-up studies OR prospective)) AND (infan* OR newborn* OR neonat* OR baby OR babies) (((TITLE(bronchiolitis) AND TITLE-ABS-KEY(steroid* OR glucocorticoid* OR corticosteroid*))) AND KEY("epinephrine" OR "albuterol" OR "corticosteroids" OR "hydrocortisone" OR "steroids" OR ("inhaled steroids") OR "salbutamol" OR "dexamethasone" OR "steroid" OR ("inhaled budesonide") OR "glucocorticoids" OR "bronchodilator" OR ("steroid use") OR "prednisolone" OR ("oral prednisolone") OR "prednisone")) AND (TITLE-ABS-KEY("Clinical Trial" OR "Clinical Trials" OR "Randomized Controlled Trial*" OR "Random Allocation" OR "double-blind method" OR "single-blind method" OR placebos OR research design OR comparative study OR evaluation studies OR follow-up studies OR prospective))	

Conference Proceedings

Canadian Pediatric Society (2004-2009)

Pediatric Academic Societies (2004-2009)

Society for Academic Emergency Medicine (2004-2009)

European Respiratory Society (2003 to 2009)

American Thoracic Society (2006-2009)

European Society for Pediatric Research (2006 to 2009)

Clinical Trials Registeres (searched November 26, 2009)

ClinicalTrials.gov

Current Controlled Trials

ClinicalStudyResults.org

Australian New Zealand Clinical Trials Registry

IFPMA Clinical Trials Portal, UMIN Clinical Trials Registry

rct zoeken – Nederlands Trialregister – Dutch Cochrane Centre

ICTRP Search Portal – World Health Organization

EBM Reviews - Cochrane Central Register of Controlled Trials – Ovid Version

OvidSP_UI02.01.02.102 4TH Quarter 2009	Searched: 25Nov09 Results: 292
1. exp BRONCHIOLITIS/ 2. (bronchiolitis or wheez*).mp. [mp=title, original title, abstract, mesh headings, heading words, keyword] 3. exp Respiratory Syncytial Viruses/ or exp exp Respiratory Syncytial Virus Infections/ 4. Respiratory Syncytial Virus$.mp. 5. or/1-4 6. exp Bronchodilator Agents/ 7. exp Adrenergic Agents/ 8. exp Glucocorticoids/ or exp Adrenal Cortex Hormones/ 9. (Glucocorticoid* or Corticosteroid*).mp. 10. exp Anti-Inflammatory Agents/ 11. exp Drug Therapy, combination/ 12. exp Epinephrine/ 13. adrenal cortex hormone*.ti,ab. 14. (epinephrine or adrenalin*).mp. 15. albuterol.mp. 16. beclomet?asone.mp. 17. betamet?asone.mp. 18. budesonide.mp.	19. dexamet?asone.mp. 20. salbutamol.mp. 21. ipratropium.mp. 22. prednisolone.mp. 23. prednisone.mp. 24. methylprednisone.mp. 25. terbutaline.mp. 26. fluticasone.mp. 27. exp Orciprenaline/ or (orciprenaline or fenoterol).mp. 28. aminophylline.mp. 29. androstadienes.mp. 30. hydrocortisone.mp. 31. or/6-30 32. 5 and 31 33. exp Infant/ 34. (Infant* or infancy or Newborn* or Baby* or Babies or Neonat* or Preterm* or Prematur* or Postmatur*).mp. 35. or/33-34 36. 32 and 35

EMBASE – Ovid Version

OvidSP_UI02.01.02.102 1980 to 2009 Week 47	Searched: 24Nov09 Results: 1717
1. exp BRONCHIOLITIS/ 2. (bronchiolitis or wheez*).mp. 3. exp Respiratory Syncytial Pneumovirus/ 4. Respiratory Syncytial Virus$.mp. 5. or/1-4 6. exp Bronchodilating Agents/ 7. exp Adrenergic Receptor Stimulating Agents/ 8. exp Glucocorticoid/ or exp corticosteroid/ 9. (glucocorticoid* or corticosteroid*).mp. 10. exp Anti-Inflammatory Agent/ 11. exp Drug combination/ 12. exp Adrenalin/ 13. adrenal cortex hormone*.ti,ab. 14. (epinephrine or adrenalin*).mp. 15. albuterol.mp. 16. betamet?asone.mp. 17. beclomet?asone.mp. 18. budesonide.mp. 19. exp Dexamethasone/ or dexametha?one.mp. 20. salbutamol.mp. 21. ipratropium.mp. 22. exp Prednisolone/ or prednisolone.mp. 23. exp Prednisone/ or prednisone.mp. 24. methylprednisone.mp. 25. terbutaline.mp. 26. fluticasone.mp. 27. Orciprenaline/ or Fenoterol/ or (orciprenaline or fenoterol).mp.	28. aminophylline.mp. 29. androstadienes.mp. 30. exp hydrocortisone/ 31. hydrocortisone.mp. 32. or/6-31 33. 5 and 32 34. exp clinical trial/ 35. randomi?ed.ti,ab. 36. placebo.ti,ab. 37. dt.fs. 38. randomly.ti,ab. 39. trial.ti,ab. 40. groups.ti,ab. 41. or/34-40 42. animal/ 43. human/ 44. 42 not (42 and 43) 45. 41 not 44 46. 33 and 45 47. limit 46 to (child or preschool child <1 to 6 years>) 48. exp Infant/ 49. (Infant* or infancy or Newborn* or Baby* or Babies or Neonat* or Preterm* or Prematur* or Postmatur*).mp. 50. 48 or 49 51. 46 and 50 52. 47 or 51

IRAN MedEx

1998 to 2009	Searched: 26Nov09 Results: 42
(Bronchiolitis or bronquiolitis or broncho-alveolites virales or bronchiolite*)	

LILACS BIREME/OPAS/OMS - Latin American and Caribbean Center on Health Sciences Information

All available years were searched	Searched: 25Nov09 Results: 224
wheeze OR Sibilancias OR bronquiolitis OR bronchiolitis OR bronquiolite [Words] and infant OR pediatric OR newborn OR nacidos OR Lactentes OR lactantes OR pediátrica [Words]	

MEDLINE® - Ovid Version

OvidSP_UI02.01.02.102 1950 to November Week 2 2009	Searched: 24Nov09 Results: 673
1. exp BRONCHIOLITIS/ 2. (bronchiolitis or wheez*).mp. 3. exp Respiratory Syncytial Viruses/ or exp exp Respiratory Syncytial Virus Infections/ 4. Respiratory Syncytial Virus$.mp. 5. or/1-4 6. exp Bronchodilator Agents/ 7. exp Adrenergic Agents/ 8. exp Glucocorticoids/ or exp Adrenal Cortex Hormones/ 9. (Glucocorticoid* or Corticosteroid*).mp. 10. exp Anti-Inflammatory Agents/ 11. exp Drug Therapy, combination/ 12. exp Epinephrine/ 13. (epinephrine or adrenalin*).mp. 14. albuterol.mp. 15. betamet?asone.mp. 16. beclomet?asone.mp. 17. budesonide.mp. 18. dexamet?asone.mp. 19. salbutamol.mp. 20. ipratropium.mp. 21. prednisolone.mp. 22. prednisone.mp. 23. methylprednisone.mp. 24. terbutaline.mp. 25. fluticasone.mp.	26. exp Orciprenaline/ or (orciprenaline or fenoterol).mp. 27. aminophylline.mp. 28. androstadienes.mp. 29. hydrocortisone.mp. 30. or/6-29 31. 5 and 30 32. randomized controlled trial.pt. 33. clinical trial.pt. 34. randomi?ed.ti,ab. 35. placebo.ti,ab. 36. dt.fs. 37. randomly.ti,ab. 38. trial.ti,ab. 39. groups.ti,ab. 40. or/32-39 41. animals/ 42. humans/ 43. 41 not (41 and 42) 44. 40 not 43 45. 44 and 31 46. exp Infant/ 47. (Infant* or infancy or Newborn* or Baby* or Babies or Neonat* or Preterm* or Prematur* or Postmatur*).mp. 48. or/46-47 49. 45 and 48

GRADE ASSESSMENTS

Strength of evidence Comparison	Number of studies	Number of patients	Outcome	Strength of evidence *	Intervention favoured
Inpatients					
Steroid vs. placebo	8	633	LOS	high	NS
	1	174	CS : 3-6 hours	low	Steroid
	3	269	CS : 6-12 hours	moderate	Steroid
	3	264	CS : 12-24 hours	moderate	NS
	4	271	CS : 24-72 hours	low	NS
Epinephrine vs. placebo	2	292	LOS	moderate	NS
	2	232	CS: 60 minutes	moderate	NS
Epinephrine vs. salbutamol	4	261	LOS	moderate	Epinephrine
	4	248	CS: 60 minutes	low	Epinephrine
	1	140	CS: 120 minutes	low	Epinephrine
Salbutamol/terbutaline vs. placebo	6	346	LOS	high	NS
	5	223	CS: 60 minutes	low	NS
	2	68	CS: 120 minutes	low	NS
	1	89	CS: 3-6 hours	low	Salbutamol
	2	136	CS : 6-12 hours	moderate	Salbutamol
	2	136	CS : 12-24 hours	moderate	NS
	3	195	CS : 24-72 hours	moderate	NS
Outpatients					
Steroid vs. placebo	8	1762	Admissions D1	high	NS
	5	1530	Admissions up to D7	moderate	NS
	4	1006	CS: 60 minutes	high	NS
	3	214	CS: 120 minutes	moderate	NS
	2	808	CS: 3-6 hours	moderate	NS
	1	69	CS: 12-24 hours	low	NS
	4	224	CS: 3-10 days	low	NS
Epinephrine vs. Placebo	4	920	Admissions D1	moderate	Epinephrine
	1	800	Admissions up to D7	low	NS
	4	900	CS: 60 minutes	high	Epinephrine
	1	30	CS: 120 minutes	low	Epinephrine
Epinephrine vs. salbutamol	6	295	Admissions D1	moderate	NS
	1	63	Admissions up to D7	low	NS
	6	148	CS: 60 minutes	moderate	NS
	4	207	CS: 120 minutes	moderate	NS
	1	69	CS: 12-24 hours	low	NS
	1	69	CS: 3-10 days	low	Epinephrine
Salbutamol vs. placebo	4	196	Admissions D1	moderate	NS
	2	259	Admissions D7	moderate	NS
	8	565	CS: 60 minutes	low	Salbutamol
	2	100	CS: 120 minutes	low	NS
	1	60	CS : 3-6 hours	low	NS
Epi+dex vs. placebo	1	400	Admissions D1	low	NS
	1	400	Admissions D7	low	Epi+dex
	1	399	Clinical score: 60 minutes	moderate	Epi+dex
Epi+dex vs. salbutamol	1	35 (no events)	Admissions D1	insufficient	n/a
	1	35 (no events)	Admissions D7	insufficient	n/a
	1	35	Clinical score: 120 minutes	low	NS
	1	35	Clinical score: 12-24 hours	low	NS
	1	35	Clinical score: 3-10 days	low	Epi+dex

LOS=length of stay; CS=clinical score; NS=not significant

* Strength of evidence assessments:[23]

High: High confidence that the evidence reflects the true effect. Further research is very unlikely to change our confidence in the estimate of effect.

Moderate: Moderate confidence that the evidence reflects the true effect. Further research may change our confidence in the estimate of effect and may change the estimate.

Low: Low confidence that the evidence reflects the true effect. Further research is likely to change the confidence in the estimate of effect and is likely to change the estimate.

Insufficient: Evidence either is unavailable or does not permit a conclusion.

A2 SUPPLEMENTARY DATA FOR COCHRANE REVIEW (CHAPTER 2.2)

LIST OF INCLUDED STUDIES

Barlas 1998 {published data only}
Barlas C, Kiper N, Göçmen A, Ozçelik U, Dilber E, Anadol D, et al.Racemic adrenaline and other treatment regimens in mild and moderate bronchiolitis. Cocuk Sagligi Ve Hastaliklari Dergisi 1998;41(2):155–66.

Bentur 2005 {published data only}
Bentur L, Shoseyov D, Feigenbaum D, Gorichovsky Y, Bibi H. Dexamethasone inhalations in RSV bronchiolitis: a double-blind, placebo-controlled study. Acta Paediatrica 2005;94(7):866–71.

Berger 1998 {published data only}
Berger I, Argaman Z, Schwartz SB, Segal E, Kiderman A, Branski D, et al.Efficacy of glucocorticoids in acute bronchiolitis: short-term and long-term follow-up. Pediatric Pulmonology 1998;26:162–6.

Cade 2000 {published data only}
Cade A, Brownlee KG, Conway SP, Haigh D, Short A, Brown J, et al.Randomised placebo controlled trial of nebulised glucocorticoids in acute respiratory syncytial viral bronchiolitis. Archives of Disease in Childhood 2000;82(2): 126–30.

Corneli 2007 {published data only}
Corneli HM, Zorc JJ, Mahajan P, Shaw KN, Holubkov R, Reeves SD, et al.A multicenter, randomized, controlled trial of dexamethasone for bronchiolitis. New England Journal of Medicine 2007;357(4):331–9.

De Boeck 1997 {published data only}
De Boeck K, Van der Aa N, Van Lierde S, Corbeel L, Eeckels R. Respiratory syncytial virus bronchiolitis: a double-blind dexamethasone efficacy study. Journal of Pediatrics 1997; 131(6):919–21.

Goebel 2000 {published data only}
Goebel J, Estrada B, Quinonez J, Nagi N, Sanford D, Boerth RC. Prednisolone plus albuterol versus albuterol alone in mild to moderate bronchiolitis. Clinical Pediatrics 2000;39(4):213–20.

Gomez 2007 {published data only}
Gomez-y-Lopez RE, Hernandez-Sierra JF, Torres-Ruvalcaba BA, Martinez-Puente E, del Carmen Martinez-Garcia M. Comparative clinical study of dexamethasone vs. nebulized salbutamol in acute bronchiolitis. Gaceta Medica de Mexico 2007;143(3):189–92.

Klassen 1997 {published data only}
Klassen TP, Sutcliffe T, Watters LK, Wells GA, Allen UD, Li MM. Dexamethasone in salbutamol-treated inpatients with acute bronchiolitis: a randomized, controlled trial. Journal of Pediatrics 1997;130(2):191–6.

Kuyucu 2004 {published data only}
Kuyucu S, Unal S, Kuyucu N, Yilgor E. Additive effects of dexamethasone in nebulized salbutamol or L-epinephrine treated infants with acute bronchiolitis. Pediatrics International 2004;46(5):539–44.

Mesquita 2009 {published data only}
Mesquita M, Castro-Rodriguez JA, Heinichen L, Fariña E, Iramain R. Single oral dose of dexamethasone in outpatients with bronchiolitis: a placebo controlled trial. Allergologia et Immunopathologia 2009;37(2):63–7.

Plint 2009 {published data only}
Plint AC, Johnson DW, Patel H, Wiebe N, Correll R, Brant R, et al.Epinephrine and dexamethasone in children with bronchiolitis. New England Journal of Medicine 2009;360 (1533-4406):2079–89.

Richter 1998 {published data only}
Richter H, Seddon P. Early nebulized budesonide in the treatment of bronchiolitis

and the prevention of postbronchiolitic wheezing. Journal of Pediatrics 1998;132 (5):849–53.

Roosevelt 1996 {published data only}
Roosevelt G, Sheehan K, Grupp-Phelan J, Tanz RR, Listernic R. Dexamethasone in bronchiolitis: a randomised controlled trial. Lancet 1996;348(9023):292–305.

Schuh 2002 {published data only}
Schuh S, Coates AL, Binnie R, Allin T, Goia C, Corey M, et al.Efficacy of oral dexamethasone in outpatients with acute bronchiolitis. Journal of Pediatrics 2002;140:27–32.

Teeratakulpisarn 2007 {published data only} Teeratakulpisarn J, Limwattananon C, Tanupattarachai S, Limwattananon S, Teeratakulpisarn S, Kosalaraksa P. Efficacy of dexamethasone injection for acute bronchiolitis in hospitalized children: a randomized, double-blind, placebo-controlled trial. Pediatric Pulmonology 2007;42(5): 433–9.

Zhang 2003 {published data only}
Zhang L, Ferruzzi E, Bonfanti T, Auler MI, D'Avila NE, Faria CS, et al.Long and short-term effect of prednisolone in hospitalized infants with acute bronchiolitis. Journal of Paediatrics and Child Health 2003;39(7):548–51.

A3 FORMULAS (CHAPTER 3.2)

1. Reliability

-Standard error of measurement (SEM):

$SEM_{agreement} = \sqrt{\sigma_o^2 + \sigma_{residual}^2}$ (o: observers)

-Smallest detectable change (SDC):

$SDC = 1.96 \times \sqrt{2} \times SEM_{agreement}$

-95% limits of agreement (LoA):

$LoA = mean\Delta score \pm 1.96 \times SD_{\Delta score}$

-Intraclass Correlation Coefficient (ICC):

$ICC_{agreement} = \frac{\sigma_p^2}{\sigma_p^2 + \sigma_o^2 + \sigma_{residual}^2}$ (o: observers, p: patients)

2. Responsiveness

-standardized/Cohen's effect size (ES) for both stable and improved groups:

$ES = \frac{mean(test_1 - test_2)_{group}}{SDtest_{1_{group}}}$

-responsiveness ratio (ReR) for the improved group:

$ReR = \frac{mean(test_1 - test_2)_{improved}}{SD(test_1 - test_2)_{stable}}$

References

Bland JM, Altman DG. Measurement error. BMJ, 1996;313(7059):744.

de Vet H, Terwee C, Mokkink L, Knol D. Measurement in Medicine a Practical Guide: Cambridge University Press; 2011.

de Vet HC, Terwee CB, Knol DL, Bouter LM. When to use agreement versus reliability measures. J Clin Epidemiol, 2006;59(10):1033-1039.

Shavelson RJ, Webb, NM. Generalizability Theory: A primer. London: Sage; 1991.

Terwee CB, Dekker FW, Wiersinga WM, Prummel MF, Bossuyt PM. On assessing responsiveness of health-related quality of life instruments: guidelines for instrument evaluation. Qual Life Res, 2003;12(4):349-362.

A4 QUESTIONNAIRES (PEDIATRICIANS AND GPS), ETHICS COMMITTEE APPROVAL (CHAPTER 4)

(only questions on definition and outcomes are shown; the questionnaire also included questions on diagnosis and treatment of bronchiolitis)

Questionnaire: GPs

Secção de Pneumologia da Sociedade Portuguesa de Pediatria
com o apoio da Associação Portuguesa de Medicina Geral e Familiar

ABBA: **AB**ordagem da **B**ronquiolite **A**guda
Atitudes na abordagem diagnóstica e terapêutica da Bronquiolite em Portugal

Caro(a) Colega,

A Secção de Pneumologia da Sociedade Portuguesa de Pediatria (SPP) está a elaborar uma norma de orientação clínica sobre Bronquiolite Aguda (BA). Com este inquérito pretende-se saber como lidam médicos especialistas e internos de Pediatria e Medicina Geral e Familiar de forma individual com a BA.
Sinta-se à-vontade para recorrer a normas que habitualmente consulta para a sua prática clínica.

Este inquérito é efectuado em nome da Secção de Pneumologia da SPP, com a aprovação da SPP e da Associação Portuguesa de Medicina Geral e Familiar. A confidencialidade das respostas será preservada.

Para avançar ou retroceder no preenchimento do questionário clique Seguinte ou Anterior no fim da página.

Se tiver dúvidas não hesite em nos contactar!

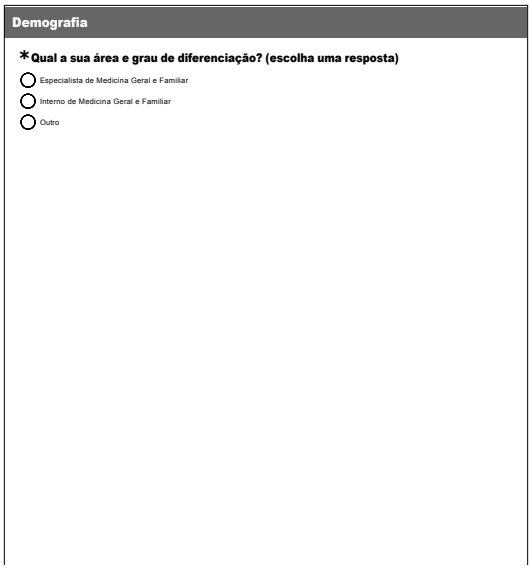
Obrigado pela sua participação!
O Grupo de Trabalho da Secção de Pneumologia Pediátrica da Sociedade Portuguesa de Pediatria

Contacto: bapneumospp@gmail.com

com o apoio

SPP SOCIEDADE PORTUGUESA DE PEDIATRIA — www.spp.pt

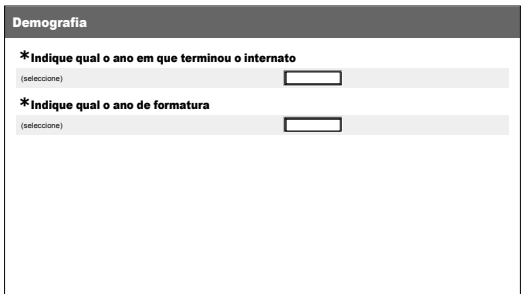
apmgf — www.apmgf.pt



Demografia

*Qual a sua área e grau de diferenciação? (escolha uma resposta)

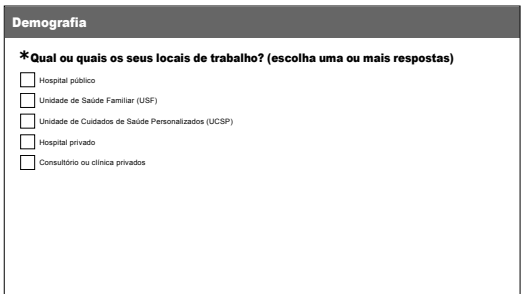
- ○ Especialista de Medicina Geral e Familiar
- ○ Interno de Medicina Geral e Familiar
- ○ Outro



Demografia

*Indique qual o ano em que terminou o internato
(seleccione)

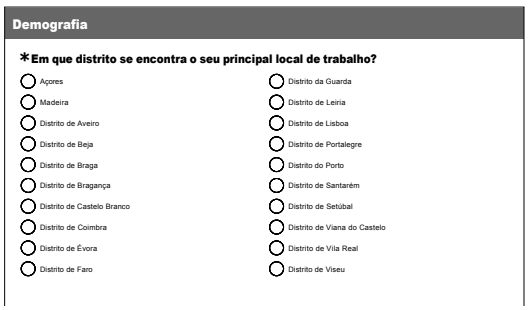
*Indique qual o ano de formatura
(seleccione)



Demografia

*Qual ou quais os seus locais de trabalho? (escolha uma ou mais respostas)

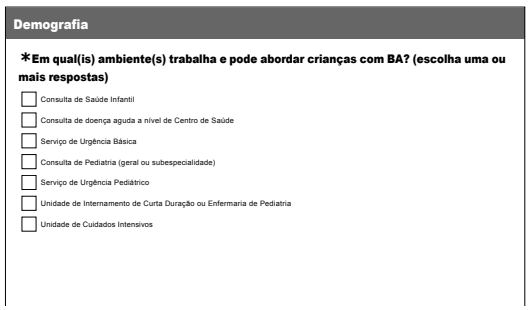
- ☐ Hospital público
- ☐ Unidade de Saúde Familiar (USF)
- ☐ Unidade de Cuidados de Saúde Personalizados (UCSP)
- ☐ Hospital privado
- ☐ Consultório ou clínica privados



Demografia

*Em que distrito se encontra o seu principal local de trabalho?

- ○ Açores
- ○ Madeira
- ○ Distrito de Aveiro
- ○ Distrito de Beja
- ○ Distrito de Braga
- ○ Distrito de Bragança
- ○ Distrito de Castelo Branco
- ○ Distrito de Coimbra
- ○ Distrito de Évora
- ○ Distrito de Faro
- ○ Distrito da Guarda
- ○ Distrito de Leiria
- ○ Distrito de Lisboa
- ○ Distrito de Portalegre
- ○ Distrito do Porto
- ○ Distrito de Santarém
- ○ Distrito de Setúbal
- ○ Distrito de Viana do Castelo
- ○ Distrito de Vila Real
- ○ Distrito de Viseu



Demografia

*Em qual(is) ambiente(s) trabalha e pode abordar crianças com BA? (escolha uma ou mais respostas)

- ☐ Consulta de Saúde Infantil
- ☐ Consulta de doença aguda a nível de Centro de Saúde
- ☐ Serviço de Urgência Básica
- ☐ Consulta de Pediatria (geral ou subespecialidade)
- ☐ Serviço de Urgência Pediátrico
- ☐ Unidade de Internamento de Curta Duração ou Enfermaria de Pediatria
- ☐ Unidade de Cuidados Intensivos

Definição de Bronquiolite Aguda (BA)

***Quanto ao padrão clínico, indique se concorda/discorda com as definições que se seguem:**

	discordo totalmente	discordo	não concordo nem discordo	concordo	concordo totalmente
A BA caracteriza-se por início súbito de sibilância	○	○	○	○	○
A BA caracteriza-se por coriza a preceder a sibilância	○	○	○	○	○

***Quanto aos achados na auscultação pulmonar, indique se concorda/discorda com as definições que se seguem:**

	discordo totalmente	discordo	não concordo nem discordo	concordo	concordo totalmente
Na BA auscultam-se sibilos	○	○	○	○	○
Na BA auscultam-se fervores/crepitações	○	○	○	○	○

Definição de Bronquiolite Aguda (BA)

Pedimos que responda às seguintes perguntas de acordo com a definição de Bronquiolite Aguda que normalmente utiliza na sua prática clínica.

***Usa o termo de BA:**

- ○ Apenas no primeiro episódio de sibilância
- ○ Até 3 episódios de sibilância
- ○ Independentemente do número de episódios

***O diagnóstico de BA pode aplicar-se em que idades?**

- ○ <6 meses
- ○ <12 meses
- ○ <18 meses
- ○ <24 meses
- ○ independentemente da idade

Outcomes

***Se fosse convidado a avaliar os resultados de um ensaio clínico de tratamento na BA, qual a importância que daria a cada um dos seguintes parâmetros (1 – nenhuma; 5- importância máxima):**

	1 - nenhuma importância	2	3	4	5 - importância máxima
Redução do risco de internamento hospitalar	○	○	○	○	○
Diminuição da duração de internamento hospitalar	○	○	○	○	○
Melhoria da dificuldade respiratória	○	○	○	○	○
Diminuição do tempo de duração da doença	○	○	○	○	○
Redução da necessidade de oxigenoterapia	○	○	○	○	○
Melhoria da tolerância alimentar	○	○	○	○	○
Evitar efeitos adversos da terapêutica	○	○	○	○	○
Diminuição do número de consultas médicas ou idas ao SU necessárias no seguimento da doença	○	○	○	○	○
Melhoria da qualidade de vida (avaliada pelos pais)	○	○	○	○	○
Melhoria da qualidade de sono da criança e dos pais (avaliada pelos pais)	○	○	○	○	○
Melhoria da função respiratória	○	○	○	○	○
Redução do risco de sibilância recorrente ou asma tardia	○	○	○	○	○

Questionnaire: Paediatricians

Secção de Pneumologia da Sociedade Portuguesa de Pediatria
com o apoio da Associação Portuguesa de Medicina Geral e Familiar

ABBA: **AB**ordagem da **B**ronquiolite **A**guda
Atitudes na abordagem diagnóstica e terapêutica da Bronquiolite em Portugal

Caro(a) Colega,

A Secção de Pneumologia da Sociedade Portuguesa de Pediatria (SPP) está a elaborar uma norma de orientação clínica sobre Bronquiolite Aguda (BA). Com este inquérito pretende-se saber como lidam médicos especialistas e internos de Pediatria e Medicina Geral e Familiar de forma individual com a BA.
Sinta-se à-vontade para recorrer a normas que habitualmente consulta para a sua prática clínica.

Este inquérito é efectuado em nome da Secção de Pneumologia da SPP, com a aprovação da SPP e da Associação Portuguesa de Medicina Geral e Familiar. A confidencialidade das respostas será preservada.

Como forma de incentivo, sortearemos 6 assinaturas da revista "Evidence-Based Child Health: a Cochrane Review Journal" - basta preencher o questionário completo e fica habilitado a ganhar!

Para avançar ou retroceder no preenchimento do questionário clique Seguinte ou Anterior no fim da página.

Se tiver dúvidas não hesite em nos contactar!

Obrigado pela sua participação!

O Grupo de Trabalho da Secção de Pneumologia Pediátrica da Sociedade Portuguesa de Pediatria

Contacto: bapneumospp@gmail.com

com o apoio

SPP SOCIEDADE PORTUGUESA DE PEDIATRIA — www.spp.pt

apmgf — www.apmgf.pt

patrocinado por

A COCHRANE REVIEW JOURNAL EVIDENCE-BASED CHILD HEALTH — http:// onlinelibrary.wiley.com

Demografia

***Qual a sua área e grau de diferenciação? (escolha uma resposta)**

- ○ Especialista de Pediatria
- ○ Especialista de Medicina Geral e Familiar
- ○ Interno de Pediatria
- ○ Interno de Medicina Geral e Familiar
- ○ Outro

Demografia

***Indique qual o ano em que terminou o internato**

(seleccione)

***Indique qual o ano de formatura**

(seleccione)

Demografia

***Qual ou quais os seus locais de trabalho? (escolha uma ou mais respostas)**

- ☐ Hospital público de referência/ central/ nível 3
- ☐ Hospital público de primeira linha/ distrital/ nível 2
- ☐ Hospital público de proximidade/ nível 1
- ☐ Centro de saúde
- ☐ Hospital privado
- ☐ Consultório ou clínica privados

Demografia

***Em que distrito se encontra o seu principal local de trabalho?**

- ○ Madeira
- ○ Açores
- ○ Distrito de Aveiro
- ○ Distrito de Beja
- ○ Distrito de Braga
- ○ Distrito de Bragança
- ○ Distrito de Castelo Branco
- ○ Distrito de Coimbra
- ○ Distrito de Évora
- ○ Distrito de Faro
- ○ Distrito da Guarda
- ○ Distrito de Leiria
- ○ Distrito de Lisboa
- ○ Distrito de Portalegre
- ○ Distrito do Porto
- ○ Distrito de Santarém
- ○ Distrito de Setúbal
- ○ Distrito de Viana do Castelo
- ○ Distrito de Vila Real
- ○ Distrito de Viseu

Demografia

***Em que ambiente(s) aborda crianças com bronquiolite? (escolha uma ou mais respostas)**

- ☐ Consulta de Saúde Infantil
- ☐ Consulta de Pediatria (especialidade ou subespecialidade)
- ☐ Consulta de doença aguda a nível de Centro de Saúde
- ☐ Serviço de Urgência Básica
- ☐ Serviço de Urgência Pediátrico
- ☐ Unidade de Internamento de Curta Duração ou Enfermaria de Pediatria
- ☐ Unidade de Cuidados Intensivos

Definição de Bronquiolite Aguda (BA)

***Quanto ao padrão clínico, indique se concorda/discorda com as definições que se seguem:**

	discordo totalmente	discordo	não concordo nem discordo	concordo	concordo totalmente
A BA caracteriza-se por início súbito de sibilância	○	○	○	○	○
A BA caracteriza-se por coriza a preceder a sibilância	○	○	○	○	○

***Quanto aos achados na auscultação pulmonar, indique se concorda/discorda com as definições que se seguem:**

	discordo totalmente	discordo	não concordo nem discordo	concordo	concordo totalmente
Na BA auscultam-se sibilos	○	○	○	○	○
Na BA auscultam-se fervores/crepitações	○	○	○	○	○

Definição de Bronquiolite Aguda (BA)

Pedimos que responda às seguintes perguntas de acordo com a definição de Bronquiolite Aguda que normalmente utiliza na sua prática clínica.

***Usa o termo de BA:**

- ○ Apenas no primeiro episódio de sibilância
- ○ Até 3 episódios de sibilância
- ○ Independentemente do número de episódios

***O diagnóstico de BA pode aplicar-se em que idades?**

- ○ <6 meses
- ○ <12 meses
- ○ <18 meses
- ○ <24 meses
- ○ independentemente da idade

Outcomes

***Se fosse convidado a avaliar os resultados de um ensaio clínico de tratamento na BA, qual a importância que daria a cada um dos seguintes parâmetros (1 – nenhuma; 5- importância máxima):**

	1 - nenhuma importância	2	3	4	5 - importância máxima
Redução do risco de internamento hospitalar	○	○	○	○	○
Diminuição da duração de internamento hospitalar	○	○	○	○	○
Melhoria da dificuldade respiratória	○	○	○	○	○
Diminuição do tempo de duração da doença	○	○	○	○	○
Redução da necessidade de oxigenoterapia	○	○	○	○	○
Melhoria da tolerância alimentar	○	○	○	○	○
Evitar efeitos adversos da terapêutica	○	○	○	○	○
Diminuição do número de consultas médicas ou idas ao SU necessárias no seguimento da doença	○	○	○	○	○
Melhoria da qualidade de vida (avaliada pelos pais)	○	○	○	○	○
Melhoria da qualidade de sono da criança e dos pais (avaliada pelos pais)	○	○	○	○	○
Melhoria da função respiratória	○	○	○	○	○
Redução do risco de sibilância recorrente ou asma tardia	○	○	○	○	○

CENTRO HOSPITALAR LISBOA NORTE, EPE | HOSPITAL DE SANTA MARIA | Hospital Pulido Valente | Faculdade de Medicina Universidade de Lisboa

Exmo. Senhor
Dr. Ricardo Fernandes
Departamento de Pediatria
Centro Hospitalar Lisboa Norte, E.P.E.

Lisboa, 21 de Agosto de 2013

Assunto: Estudo **ABBA "Abordagem da Bronquiolite Aguda"**

Relator – *Dra. Elisa Pedro*

Pela presente informamos que o projecto citado em epígrafe obteve, na reunião em 27 de Março de 2013, parecer favorável da Comissão de Ética, pendente da aquiescência do Director do Serviço envolvido para a sua realização, presentemente anuída.

Com os melhores cumprimentos,

O Presidente da Comissão de Ética para a Saúde

Prof. Doutor João Lobo Antunes

COMISSÃO DE ÉTICA CHLN/FML
Secretariado: Ana Cristina Pimentel Neves e Patrícia Fernandes
Tel. – 21 780 54 05; Fax – 21 780 56 90
Av. Professor Egas Moniz
1649-035 LISBOA

www.chln.pt

Alameda das Linhas de Torres, 117
1769-001 LISBOA
Tel: 217 548 000 – Fax: 217 548 2